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A PUBLIC MEETING

SALMONELLA ENTERITIDIS RESEARCH

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FOOD AND DRUG ADMINISTRATION

A PUBLIC MEETING

SALMONELLA ENTERITIDIS RESEARCH

Ballroom
Holiday Inn Crowne Plaza Hotel
1325 Virginia Avenue
Hapeville, Georgia

Friday, September 8, 2000
8:30 a.m.

ROBERT BRACKETT, FDA, Presiding

P R O C E E D I N G S

MR. BRACKETT: Good morning. Welcome to this public hearing. We have people here to address the research that is being done on this problem, and we have some who represent other interests.

This relates to the background in *salmonella enteritidis* illnesses that have increased over the past decades, and to the point where in your package you also have the Egg Safety From Production to Consumption Egg Action Plan, and this was published in 1999 as the long-range strategy to address this issue.

One of the -- there's a number of different objectives that are outlined in the action plan, but specifically one that we are interested in is research, that is how do we get the information that we need to make the policies and the decisions that we need to do to solve this problem.

And the specific areas which also are listed on your agenda is that there were four very broad objectives to this, and as I said they're on your agenda, and each of our speakers or group of speakers have been asked to sort of summarize and address what has been done and where things are going in these specific areas.

The specific topics range all the way from very applied, very on-farm practical type research all the way to molecular and genetic methods that would help us get to the mechanism of *salmonella enteritidis* illness in animals as well as in humans, and so it really spans the whole scope of what could be done in biological research.

As I mentioned, each of the speakers will provide sort of an overview, so this is by no means a comprehensive discussion; it is meant to sort of identify the gaps, and really that is the goal of this meeting, and what we hope to come away with at the end of the day, and that is to address sort of the state of the science regarding SE.

There have been many symposia over the years that have addressed SE, but this one is a little bit different than others in that we are specifically addressing those research items that were addressed in the action plan. And so the idea is to find out where we are right now, that is what has been accomplished that's in the action plan, where things are going right now -- we hope to hear a little bit about what research is going on now that perhaps has not been published yet, and more importantly to identify those research gaps so that we can find out what needs to be yet done in the future.

And so the outcome of this meeting, that is the research gaps, finding out what has been addressed will help to set, or at least allow both regulatory agencies as well as industry to focus their research dollars in a more effective way. That is identify the research gaps and set funding priorities as well as perhaps readjust the priorities that have already been set.

The format that we're going to use today is a little bit of a mixture of a variety of different techniques. The first groups addressing the different goals will be sort of symposium style, that is the speakers will give an overview; we will allow a few minutes if possible for technical questions, and we do ask that you limit these to technical questions. If you have other opinions or other questions, please wait until the end of the day during the public comment period.

Secondly, in the afternoon we will have a panel discussion with the speakers, and the goal of this is to get the speakers to answer some of the questions that were also identified in the Federal Register notice, which is to figure out what research and consensus looks like needs to be done

-- is where are the research gaps -- and perhaps some other questions, for instance what is the best way to get this research done. That is, who is to fund it, is it best done through private funds, is it best done through government funds; if so, how should that be done. Would it be best as a competitive grant? Would it be best as contracts? These are the sorts of questions that we would like to get some input on.

And then finally at the end of the day we will have a public comment period in which each person who wishes to can give a five-minute statement, or if they have written comments they can provide those also to Wendy Buckler.

Wendy Buckler for those of you who have not yet met her is the lady standing in the doorway, and the person who is really the person that gets the credit for organizing the meeting, and she will handle all of the audiovisuals for the speakers, as well as getting the information to the dockets.

Now, since this is a public meeting all of the comments will be recorded, and it will be part of the public record, and so anything that is said here has to be available to the public, and so during the public comment period that's why there's only five minutes, and if people have more to say they can send in written comments as well.

Finally, a little bit about the hotel. If you haven't already found them, the restrooms are all the way down the hall out the door to your right, and we will take several breaks, and we hope to keep those short and on time. And then also we will break for lunch. We are going to try to get a list of restaurants that are nearby. There are some right in the hotel here, there are some within walking distance although it's raining, and if you have a car there are some others just down the street, but there are a number of restaurants within five-minute drive, and some within a walk.

Okay. At this time I would like to also acknowledge the help that we've had from the Agricultural Research Service in their providing speakers, as well as the Food Safety and Inspection Service for helping to organize this. This has been a very cooperative effort that affects all of us, and so we try to do this in a concerted effort.

Okay. I guess we'll get started here. Our first speakers will be Peter Holt and Bailey Mitchell who are from ARS. They are ARS scientists who are specializing on *salmonella enteritidis*, and they are going to first address Objective 7, that is to ensure adequate current information is available to make decisions, but specifically 7.1, to develop and evaluate on-farm intervention strategies and technologies, and they are going to split their time, and first we'll have Peter Holt speaking.

STATEMENT BY PETER S. HOLT, SOUTHEAST POULTRY RESEARCH LAB, ATHENS, GEORGIA

MR. HOLT: Thanks Bob.

Bob has had me do the Objective 7.1 which is to conduct research, to develop and evaluate on-farm intervention strategies or technologies.

There's a lot of information to be given, so what

I'm going to have to do is go fast and furious through a lot of it to get through everything, and rather than the long of it I'll give the short of it.

The first part of the Objective 7.1 is forced molting and other stress factors. The question that occurs is why molt in the first place.

Now, as a laying flock ages its ability to lay eggs decreases, and it reaches a point where it's no longer economically feasible to keep the flock in lay. A producer can send all his birds to slaughter and bring on a new flock, or he can recycle his birds.

Well, what the general trend is is most of the producers recycle their birds. This is a slide from 1987, and about 60 percent of the flocks were recycled at that time, and it's moved up to about 70 percent now.

When you put pen to paper figuring about 240 million birds in the U.S. that comes to somewhere between 144 and 168 million birds that are molted annually.

Now, there's a reason for this. Most of the early studies have shown that the effects of molting were primarily positive, that it increase productivity. Of course, that's the reason they recycle the birds in the first place.

Increased feed conversion, and actually on a number of the studies they actually had less mortality than their unmolted counterparts, but that's not always the case, so this equaled the rest of the rigors of daily egg lay.

Now, there are a number of ways to molt birds, but feed and nutrient restriction and feed removal are the two prevalent procedures to recycle the birds, and feed removal as shown in the green is the procedure that we looked at, and this is the primary procedure that we worked with. Generally dropped the photo period down to eight hours a day because egg lay is affected by photo period; take the birds off of feed and that drops our particular flocks' weight somewhere between 25 and 30 percent, and then start them back on the grower ration throughout the experiment.

Now, the first thing we looked at was the effect of molting on immunity, and we found that there were some pretty dramatic effects. While humeral immunity to antibody response was largely unaffected, cell mediated immunity was significantly depressed as indicated by three different parameters, and when we did photositometric analysis of the peripheral blood lymphocytes we found that the CT4+ T cells, the helper T cell subset was significantly decreased.

Now, the importance of the immune system is severalfold. First of all, in order to elicit to vaccination you need an intact immune system, but in birds this age that really doesn't play as big a factor.

Where it does play a factor is it affects their ability to fight disease, whether it be viral, protozoan, fungal, or bacterial, and so we focused in on a bacterial infection which is *salmonella enteritidis* and we found that molting did have a substantial effect on experimental infections, and I need to stress that that all the SE studies that we did were all experimental, done under controlled conditions with our specific pathogen-free flocks.

But birds that were infected during the molt, we had increased shedding, birds were infected for longer periods of time. If we infected the birds before the molt normally the normal-fed birds would generally clear the infection, but the molted birds a certain percentage of them would stay persistently infected, and that's shown in this slide.

You can see in the unmolted birds shown in green by day 24 they had essentially cleared the infection, but you can see that a certain percentage of the molted birds stayed positive throughout the experiment.

Molting also affected the susceptibility to infection. Generally it takes somewhere around five times ten to the fourth SE to infect a bird; it takes less than ten during the molt. So they're extremely susceptible to infection at this time, and because of that you get a very rapid horizontal spread to uninfected hens in adjacent cages.

And the way we ran this experiment is we had cages of molted and unmolted birds, eleven birds per row, and we infected just the center bird with a dose which is right around fifty percent of the infectious dose for unmolted birds, and you can see in the red that the unmolted birds had very little transmission; the molted birds you got a very rapid transmission. By day three about 35 percent of the birds were positive, and by day ten it's 85 percent, and they remained high from then on.

Now, all these studies were done in experimental conditions. There have been some studies looking out in the field, and this is from the SE pilot project, and they looked at the production of SE-positive eggs, and they did find that weeks zero to five post-molt there was an increase in the production of SE-positive eggs, and I think, Eric, you will probably be talking a little bit about that as well, so I won't dwell on it.

Now, what might be some of the causes that are affecting the SE infection. Immune depression is probably very prominent, but we saw on occasions effects occurring within 24 to 48 hours after infection, which is awfully fast for effects on specific immunity to play a role, so it had to be other factors, and depression of the immunity cropped up as a potential possibility, and Dr. Mike Cogan with the

USDA lab down in College Station, Texas showed that heterophil function, the white blood cells were significantly depressed, so it looks like immunity is affected.

We thought because the birds were off feed that there would be an alteration of the intestinal microflora, and Dr. Don Coyer also from the lab at College Station, Texas, and unfortunately has recently passed away couldn't find any effects on the gut flora. It doesn't mean that they aren't occurring, it just means that they couldn't find them.

And finally there may be an effect on peristalsis and digesta. The combination of peristalsis and digesta are very effective in keeping the intestinal tract clean, and by removing the feed you very well may be eliminating one of the protective capacities.

Now, for some of the solutions, looking at the effect of digesta we ran a number of different what I call alternative molt procedures, molting the birds alternative to total feed withdrawal, and working in collaboration with the scientists at Poultry Science Department at University of Georgia they developed a low-energy/low-calcium diet which we then ran in comparison with total feed withdrawal, and we found that while the shed rate was largely unaffected, and that's a trend we normally observe, the amount of SE that's shed is significantly decreased, and that this is very important for transmission to other birds, for disinfection and cleanup in the house, and also for contaminating rodents and flies in the house as well.

Now, this experiment used a metered amount of feed. We generally gave them sixty grams per day, so that does make it a little bit more difficult for the producer, and the procedure never caught on.

We also looked at low nutrition/lower energy feed additives. Soybean hulls an cracked corn really didn't work all that well. We did see a decrease in the amount of SE being shed, but where we really saw effects were with what middlings, and wheat middlings are a byproduct of what processing.

And when we gave the birds *ad lib* amounts of wheat middlings we saw a very substantial decrease in the amount of SE being shed, actually back down to control levels.

I think very telling is the amount of SE that's disseminated extraintestinally, either the liver and spleen or the ovary, and actually with the ovaries we couldn't find any SE in the two fed groups, but 63 percent of the birds were ovary positive in the total feed withdrawal.

Now, the whole point behind the research is to try and find intervention strategies that may help on the SE infection, so we also looked at antibiotic therapy, and I'm saying right now I'm not an advocate for antibiotic therapy, but I thought it was important to look at it.

And working in collaboration with Baer Corporation we looked at the use of Baytril, an antibiotic, and eliminated the SE infection, and what we did was we administered the Baytril after the birds had finished up the feed removal period, and then after the ten-day regimen of Baytril when we put them on AviGuard which is their competitive exclusion culture to repopulate their intestinal tract.

And what we found was is that the Baytril did substantially decrease the percentage of birds that were SE positive in 33 to 4 percent by day 33, and from 25 percent down to zero percent by day forty. So it can be an effective way of eliminating SE infection after a molt.

And finally vaccination. Now, we worked up a collaboration with Megan Health using their live *salmonella* vaccine as a protection, potential protective capacity. This was requested by Gene Gregory from United Egg Producers to see what effect it would have, and what we did was that we vaccinated the birds two times with the Megan vaccine by aerosol two weeks apart, and then two weeks after the second boost, and challenged the birds.

Using the transmission study that I talked about before we had our groups of molted birds, and the center hen in each row got three times ten to the fifth SE, and then we followed the transmission down the line.

Now, this is day three post-challenge, and with the non-vaccinated birds we had about 25 percent of the birds were SE positive by day three; only 5 percent, one bird in the vaccinated group.

By day ten 75 percent of the birds were SE positive in non-vaccinated as opposed to 45 percent, but what you can look at is in that 45 percent it's very low numbers as opposed to like ten to the fifth in some birds, ten to the third, so the unvaccinated birds were also shedding substantial amounts of SE as well.

By day 17 the birds are starting to clear, but there are certain birds that are still shedding quite a bit of SE in the nonvaccinated group, and as far as internal organs go, the vaccination totally eliminated any extra-intestinal dissemination to livers and spleens or to ovaries.

So where do we go from here on molting? There is quite a bit that needs to be done. I think the wheat middlings show an awful lot of promise. I think that there are probably other possible procedures that need to be looked at, and once we settle on one we need to kind of determine just the total effect on the SE infection, looking at the 50 percent infectious dose pathology itself, et cetera.

Also more work needs to be done on molt as a stressor, and we have worked up a collaboration with a relatively new USDA lab, the Livestock Behavior Research Unit at Purdue, to look at the effect of molting on various neuroendocrine factors and behavior, and so what we plan on doing is once we get the initial studies with feed withdrawal done we'll start looking at the alternative molt procedures as well to see just how much of a stressor that is.

And last, but not least, is examine molt against SE in the field, and I really think this is an important variable. There has been very little work really done out in the field looking at the effect of molting on SE, but at the same time an awful lot of verbiage has been made about molting as a possible food safety situation, and the only way that this question could be put to bed is to actually go out and look at it, and that's what we plan on doing.

And what we want to do is go out and follow SE infections in flocks from before the molt, during the molt, and afterwards, and then look at a number of different parameters which may affect health science -- age of the flock, manure handing, and see if there is one or two or several different parameters which may enter into the equation.

And this is actually the number of the parameters we want to look at, and other *salmonella* -- and I'm going to have to thank Doug Waltman for this suggestion -- this very well may be a very important parameter, and not a negative parameter, a positive one that the presence of a number of *salmonella* very well may offer some degree of protection.

Now, there has been some work out in the field, the SE pilot project that I mentioned before, and also the NAHMS which are connecting the incidence of SE in houses with the molting procedure. Previous status of the house is unknown, so it's totally an epidemiological situation. And this is the questionnaire in kind of a nutshell in the NAHMS study.

Now, also in that Objective 7.1 is other stressors in SE. There has not been a lot of research that has been done. Disease is kind of the primary one. Phillips and Opitz showed in 1995 that infectious bursal disease increased the persistence of SE infection in birds and the number of SE-positive eggs.

Qin *et al* over in Japan -- this is a Japanese group that has done just a tremendous amount of work on coccidia and the effects on SE -- there have been a number of studies on environmental stressors, thermal, crowding, transport on *salmonella* infections in general, but nothing specifically on SE, and intoxication which generally would be like microtoxins, aflatoxins, T2 toxins that has also been known to affect *salmonella* infections.

Okay. The next intervention strategy would be vaccination and its effects on *salmonella enteritidis* infections. There are two primary types of vaccines. There are multiple different kinds of vaccines available, but the two primary ones that are available commercially are live which are attenuated *salmonella* which reduces the effectiveness for the host and for humans, and it's generally administered in the feed, water feed, or possibly as an aerosol, and inactivated which most everyone is familiar with, your standard vactarins which are injected.

As far as the live vaccines go, there is only one available commercially licensed in the United States, and that's Megan Vac from Megan Health, Incorporated, that's a double-dilution mutant, it's a cyclic AMP, a cyclic AMP receptor protein mutuant.

There are a number -- and this is just a small number of live vaccines that are out and available -- Zoosaloral, Zoosaloral H, and *Salmonella* vac T out of Germany. Fort Dodge is working with an Aral A, and there is a rough strain of *salmonella gallinarum* that was developed by H. William Smith back in the 1950s that's floating around.

There are currently three *salmonella* bacterins licensed in the United States, Layermune SE from Biomune of Lenexa, Kansas; Maine Biological Laboratories has an Inacti/Vac SE4; and Fort Dodge has recently come out with one Poulvac SE; and for those individuals who want to clear up their *salmonella* infections in their flocks there are autogenous vaccines that can be made by these companies as well.

Now, inactivated vaccines have worked pretty well in clearing up experimental infections, reduces clinical science and pathology, shedding is reduced, organ positivity, the A-positivity, studies showed that growth in egg contents was reduced.

The problem is vaccination can't be used in and of itself, it has to be used in combination with good management practices to help eliminate the SE problem in the flock.

Field work, most of the studies that come from the SE pilot project saw some reduction in positive environmentals and positive eggs. The Pennsylvania Egg Quality Assurance Program has showed that there was a substantial decrease in environmentals, and the eggs from environmentally positive eggs were 8 percent positive which were reduced to zero percent positive, so it does look like vaccination very well may have a role in reducing SE problems in the field.

Particularly telling is the inactivated vaccine in England. The producers over there, about 80 percent of them signed up to vaccinate their birds, they used a vaccine produced by Hoechst which was an iron-starved *salmonella enteritidis* which produces some iron scavenging proteins which they felt would be effective in a vaccine. They vaccinate the birds at hatch, and then when they are transferred to the layer facility, and they have seen a pretty substantial drop in *salmonella enteritidis* cases, and they feel that vaccination has played a very substantial role in that.

And protection by live vaccines, there has not been a lot of field data on live vaccines. It's still too new. This is experimental data, and essentially shows very similar results than the killed bacterin. There has been some observations of cross protection against different *salmonella* serovars, but that is variable with the vaccine, and as with the other -- with the bacterins this can only be, it needs to be used with good management practices.

What are the future directions for that? I think that we're going to see more live vaccines coming on the scene, and I would love to see them. I think live vaccines are a very important mechanism for helping to eliminate the SE problem.

Mucosal vaccinations, before I was redirected back into molting we had an active group going in that, and I think that can have a very major role in the future as well.

In ovo vaccination very well may play a role, and we've had some promising results from that as well.

And subunit/vectored vaccines and DNA vaccines are down the road.

Finally one last intervention strategy is competitive exclusion. The whole principle behind competitive exclusion is that very young birds lack an intact flora first week post-hatch, and Nurmi and Rantala in 1973 showed that if you took intestinal contents from adult birds and gave them to these newly-hatched birds it would help protect against *salmonella* infections, and there have been a number of studies that have shown it's been very effective to prevent colonization of chicks with different *salmonellae*, including *salmonella enteritidis*.

Now, what role does competitive exclusion play for SE? Just a partial role actually. It can be very important in preventing colonization in newly-hatched chicks, and this can be really important.

Richard Gast and I have done some studies where you infect very young birds, and they generally a lot of times will develop a persistent infection that lasts all the way out into egg-laying, so it's very important to try and clear up that infection as early as possible.

It has fairly limited utility in adult birds because they already have a well-developed intestinal flora. However, if the birds have been subjected to antibiotic therapy, then you can use competitive exclusion to repopulate the intestinal tract.

And finally there is only one commercial competitive exclusion product available or licensed here in the United States right now, and that's Pre-empt from Milk Specialties, but there are several other commercial products that are available, and hopefully the license will be approved in the not too distant future, Aviguard from Bayer AG, and Broilact from Farmos Orion.

The Poultry Microbiological Safety Research Unit in Athens, Georgia has also developed a mucosal competitive exclusion, and they are working for licensure as well.

And saccharomyces boulardii is actually not really a competitive is not really a competitive exclusion, it's more of a sponging type of organism which actually causes the *salmonella* to adhere to their surfaces, and then they just pull them on out of solution, or out of the intestinal tract. And that's it. And what I'll do is go ahead and pass the baton over to Bailey Mitchell who will be talking about negative air ionization.

STATEMENT OF BAILEY MITCHELL, USDA-ARS Southeast Poultry Research Laboratory, Athens, Georgia

MR. BAILEY: I want to look at a little different approach. From an engineering perspective there's also some things that we could probably do intervention-wise in dealing with SE. I basically want to go over some possibilities with electrostatic space charge.

Basically what I want to do in this approach is to reduce SE levels in the air by removing bacteria-laden dust, and there's also some killing effect that we might be able to use.

The results that we're looking for is to basically reduce SE transmission between birds, houses, poultry areas, and also to reduce SE-contaminated eggs, and cross-contamination, also a good potential for improving bird and animal caretaker health by improved air quality.

Basically what we're trying to do is introduce a strong electrostatic charge into an enclosed space. This will charge any kind of dust or particulate matter in the air in a negative direction, and then that dust would be attracted to room surfaces, or if you have in some cases specialized collectors that collect this dust off.

An interesting thing here, you can get a little extra bang for the buck by taking dust out because there have been some studies done that show for example if you take out half the dust in a room by various means that you can reduce airborne bacteria by a factor of a hundred or more, so a lot of bugs attach to dust.

Just a little quick video here in a small ionizer chamber, a small hatching cabinet, with the ionizer off you can see the smoke source just kind of dissipating here.

This is with it on, it's drawn to that grounded plate there.

A little closer up view with the ionizer off. That's on.

That just gives you a little visual picture of what you can do. You can draw materials for a foot or so in that manner.

This is looking at some feathers just to see what you can do with feathers, something that large. They come down through a tube that's got a grounded strip on the right without the ionizer. This is with coming up next. You see that stuff being drawn over to the ground strip on the right side.

We did some work in a room with caged layers, put an ionizer unit in the center of the room, and we had exhaust filters in the back that you can see here that are normally blue when they're clean. In this case the birds were infected with SE, mature laying hens. We ran the experiment for about ten days, and we found we were able to reduce the dust level by 52 percent with the ionization compared to an identical room without.

Notice after ten days this filter on the exhaust still looks basically clean on the ionizer room; the other room is starting to plug up with the chicken dust here.

Interestingly, right after that we ran the SE experiment and looked at SE levels in the air with plates spread around the room, and ran that for ten days with 24-hour samples, and found we reduced airborne SE by 95 percent, so that kind of reaffirms this concept that if you take dust out you'll get a little extra benefit on your bugs.

Another interesting study here, some folks in England looked at various ways of getting *salmonella* into eggs with *salmonella typhilurium*, and using an oral challenge they were able to get about 2 percent positive eggs. With the aerosol challenge, low-level aerosol they were able to get about 14 percent. That's about eight times more than the oral challenge.

With a little bit higher aerosol they were able to get 25.4 percent. That's about 15 times more than the oral challenge, so it does kind of suggest that the aerosol route is important more than probably a lot of folks might have thought.

We did some stuff, Dr. Gast and I did some studies with looking at airborne transmission in some special cabinets where we could isolate donor birds in the front part of the cabinet, air flowing from front to back, put susceptible birds in the back, and we started out with day-old birds up here, inoculated them with SE, and then we look at the transmission downwind.

Just to look at the results at day ten, surface contamination in the untreated -- I didn't say that, one of the cabinets had an ionizer in it and the other one didn't -- in the untreated cabinet there was a hundred percent surface contamination, cecal contamination about 30 percent, and then over here -- well, I'm sorry -- this is surface contamination on the treated birds, and then cecal contamination about 90 percent on the untreated birds, and we had none here at ten days on the treated cabinet, so it had a good effect on airborne transmission as indicated by surface, particularly by cecal contamination.

We put these things in some commercial hatching cabinets also. This is a Jamesway cabinet, you can see the ionizer units here, they go on both sides of the fence. We put a grounded collector plate on each side. You can see it a little closer here, just a series of electrodes with high voltage DC applied to it to generate the ions.

Look at exhaust covers just to get a sense of the visual effect. After a hatch this is an exhaust cover from an ionizer cabinet. You see it looks quite clean here versus the control cabinet without any treatment. You can tell quite a difference there.

We were doing some plate sampling, auger plates. This a control cabinet in the upper part of the exhaust. This is the upper part of the exhaust on the ionizer cabinet, so I think you can see we're getting a good dust reduction.

We have also done a lot of plate samples using things like XLT plates, McConkey plates, and exhaust of hatching cabinets. In this case these were some XLT plates with the treatment cabinet with the ionizer versus a control cabinet without, so we're looking at ecol-I, maybe some *salmonella* here.

This is with the higher flow rate. You can see it's a little more dramatically on the treatment versus the controls, so we get usually somewhere in the neighborhood of 95 percent reduction in airborne pathogens by using this process in the hatching cabinet.

We have also done some studies just to look at the potential inactivation effect of this electrostatics. We have used, in a safety cabinet used a little chamber here with Argo plates in there, XLT plates, pump some air through a solution containing SE, pump that aerosol into the chamber, we've got a small ionization unit in there, and we look at how much SE we can recover with and without the ionizer.

I'll show you the results of the individual plates, but we go in and rinse everything out, take a sample of that rinse, we get something like this typically with a control plate it's all SE. There's the treatment plate.

So it looks kind of encouraging. We don't know exactly what level of charge it takes to get that, but that's a pretty high charge level. That's the next thing we need to look at is what kind of charge level it takes to get that.

We've also done some biofilm studies with Judy Arnold over at the Russell Center using broiler carcass rinses, taking a cocktail off of that, putting it on stainless steel coupons and treating those with electrostatic process. We got 99.8 percent reduction in three hours, 97.3 in two hours. This is consistent, and so that looks kind of encouraging as a potential non-chemical sterilizing technology that could be applied to SE as well.

Just something to give you a little relevance for this stuff. We did get recognized last year for tech transfer with the technology. It's also been listed in the President's Egg Safety Action Plan, it was listed as ion air scrubbers in hatchers. I would suggest a more appropriate name would be electrostatic space charge; it's not just a hatcher type thing.

Just like air quality, if you can clean up air it doesn't matter, you can do the same thing in a lot of places other than just hatchers.

Basically the system has been patented, it's been licensed to a company for manufacture and distribution. We've done a number of trials, commercial trials with it with commercial broiler folks, and we've got about three other commercial trials in progress. Still doing things back at the lab in the research setting.

Basically application areas would include continue to look at this inactivation process, airborne and surface SE. We've got a proposal pending on that.

We want to look at and see what we can do in a breeder house setting where you're feeding a lot of this material into the hatcher. We've got a grant proposal pending on that.

And then depending on how that goes we might want to look on out at production house, egg rooms, and we're already looking at hatching cabinets.

That's it.

MR. BRACKETT: Thank you Peter and Bailey.

We do have a couple minutes for any technical questions if there's something that either of the speakers did not make clear. First of all, if you do have questions we're going to ask you to go to the microphone and state your name as well as your affiliation for the record. In the meantime our next speaker is preparing his presentation.

Do we have any questions for either Dr. Holt or Dr. Mitchell?

[No response.]

MR. BRACKETT: Okay. Our next area of interest of course is Area 7.2 in the action plan, and that is to conduct research and provide additional information on commercial processing technologies and practices, so this goes from the realm of the farm now to food processing and more into food technology.

There are a number of investigators that are looking at this around the country. This morning we have with us Dr. Ahmed Yousef who is on the faculty in the Food Science Department at Ohio State University, and he will be providing an overview of some of the food technology type applications.

STATEMENT OF AHMED YOUSEF, Ohio State University

DR. YOUSEF: I will be talking about current and potential processing technologies and egg safety, so I will modify the topic a little bit.

Egg processing and safety, you can deal with two types of products, shell eggs and liquid whole eggs, but frankly because of time limitation I will focus basically on shell eggs, their safety and the processing and how the processing techniques affect the safety eggs.

The microorganisms of concern in shell eggs, of course we know that *salmonella* is one of them, but we know that other pathogens also can be important in shell eggs like microplasma viruses, and nonpathogenic microorganisms like pseudomonas proteus and even molds can be a problem.

With liquid whole eggs of course *salmonella* coming from shell eggs, but other microorganisms may be found in whole eggs that were not found in shell eggs like conceomoctogones [ph] and other gram negatives and spore formas which affect the quality of liquid whole eggs.

And the processing techniques that are meant to deal with microbial problems of shell eggs or liquid whole eggs include washing, in-shell pasteurization, or some alternative technologies that are coming up these days. These alternative technologies are not in practice, but they are coming pretty strongly, and I will comment a little bit o some of these.

I'm sure all of you know that *salmonella* gets into eggs through one of these three routes: if the ovary of the hen is infected, then there is a good chance that the egg coming from that ovary will be also containing *salmonella*. And the pathogen stays in the yolk in this case, and there is a chance for growth of the pathogen inside the yolk. However, trans-shell infection can happen. We call this horizontal, sometimes we call it horizontal. This happens through fecal contaminants. While the egg is being laid feces can be on the outside shell, and these may get sucked into the egg and contaminate the interior, the inside contents.

Improper washing may aggravate this problem, and the pathogen stays most of the time in the shell, but it may migrate trough the white and may eventually actually reach the yolk.

During egg-breaking if the shell is contaminated there is some chance of course that the pathogen will end up in the eggs.

So I will focus a little bit on processing shell eggs and how this affects the safety of the egg. These are the reasons that I think people should keep in mind while they are processing shell eggs. Of course, washing is done for visual reasons, aesthetic reasons, but freshness, shelf life, and the safety against external infection and internal infection should be in the minds of processors who are introducing new technologies.

So washing is done basically to remove fecal matter; this is the primary reason for washing. In fact, in some European countries they don't wash eggs, and they consider that washing is making eggs unsafe.

It all depends. This is a typical commercial egg washing process here. From the henhouses eggs are transmitted by a conveyor belt to the washing machines where the eggs are dipped in tanks containing chlorinated water and detergent. Usually the pH is pretty high, sometimes ten, sometimes eleven, and the temperature is mild, 110 degrees Fahrenheit, and this happens very quickly, one to two minutes.

Then the eggs are rinsed in hotter water, 140-150 degrees Fahrenheit for five seconds, very quick, dried with air because you want to remove as much water as you can, five to seven seconds, and then the eggs are candled, graded, packaged, and most importantly refrigerated during storage, because it has to be refrigerated at less than or equal to 45 degrees Fahrenheit, and goes through distribution.

The chlorine concentration, of course there are many variabilities in these washing operations, and people using different concentrations of chlorines, different temperature profiles, but we should understand that if we just soak an egg in water, a freshly-laid egg in water, we can be dissolving pathogens or fecal matter that may contain pathogens, and basically driving these pathogens into the egg through the pores in the shell.

Of course the regulations now, they inspect fecal matter from henhouses and should be free from *salmonella*, and if it isn't usually they follow up with certain actions.

So what washing is doing to the goals I just mentioned: For aesthetics, yes, it does remove visible fecal matter from eggs; freshness I would say questionable; shelf life probably; but egg safety I don't think this process really contributes much to egg safety, whether it is external infection or internal infection.

In-shell pasteurization came to take care of the infection problem, especially internal infections. The industry would like to define in-shell pasteurization as a precisely-controlled conductive thermal process, processes designed to effectively address *salmonella* egg safety concerns. They define that as at least 5 log degrees in the count of *salmonella*, while maintaining the appearance, texture, and functional characteristics of fresh high-quality shell eggs.

How this was developed originally, that is the patent that resulted in in-shell pasteurization, or one of them, basically they were inoculating the eggs with *salmonella enteritidis*, and initially they were really inoculating the eggs outside the yolk.

If you reach with the inoculum inside the yolk you usually puncture that membrane, and there may be a problem. So they stayed just outside the yolk and inoculated there. Later on, subsequently they did inoculation into the yolk, but this is originally how it was done.

Then eggs went through certain water bath at different temperatures. The temperatures they used, they are 56 to 60, and kept it at different times until they felt confident that they can reduce up to five logs, and they checked the produced eggs for counts of *salmonella* and quality like pH and other properties.

Now the process is practiced this way: They transfer the eggs to a pasteurizer, preheat, and that takes some time. The eggs are staying in water until they reach the hold temperature. Then once the internal temperature is about 56, they keep these eggs there anywhere from thirty to forty-five -- it should be thirty to forty-five minutes, I apologize for the mistake on the transparency. That translates to about a five-log reduction, then they are cooled, and the rest of the process. So it is a lengthy process, and it involves keeping the eggs in water for a long time.

How in-shell pasteurization meet these goals that I mentioned earlier. For aesthetics of course it will remove fecal matter and other problems. For freshness it has been claimed that it is close enough to fresh eggs, or nonprocessed eggs. Shelf life probably will improve. But the safety is the real concern, and we know that this can take care of internal infections, and of course it can take care of external infections. Heat does work, we know that.

Then there are alternative technologies that I would like to spend some time on that, still talking about in-shell processes.

Ozone can be used. Pulsed light, there is a technology there where they can pulse flashes of light to the eggs. These flashes are about 20,000 times the intensity of sunlight, and after a few flashes you can reduce the population, the internal population of *salmonella* in the egg white more than five logs, so it's pretty promising. But nobody really knows the quality of the eggs coming out of that. There's only one company, or a few people who are really playing with this. It's very hard to come up with equipment that you can test it and verify it.

Irradiation has been tried. It does work, but research shows the quality of the eggs are not that great.

High pressure, talking high pressure technology is coming up. We are talking about pressurizing things up to a hundred thousand psi or even more, and the engineers that I work with have convinced me that at hydrostatic pressure you can put an egg in there and it stays intact. I didn't believe them, and I tried that; unfortunately all the eggs cracked. They blamed it on the air cell inside the eggs, but I know that some others probably have tried it and succeeded.

The eggs that were contaminated with *salmonella* that has been high pressurized, they came out free from *salmonella*, but they were half cooked because the process also is high-pressure, nonthermal, but it does produce alterations in the properties of eggs.

Combination treatments are very promising. It's very nice to combine heat with something else. Since we know heat works, then you can use it at less intensity, but you combine it with other factors.

I'll spend a little more time on ozone since this is the work I have been doing over the past four or five years, and we would like to call this cold sanitization of shell eggs. We don't call it pasteurization because we know that we cannot really pasteurize eggs with a sanitizer, a strong sanitizer like ozone.

Ozone as you know is as natural as rain and thunderstorms. In fact, this is what you smell after thunderstorms because of the freshness of rain, and you smell it all the time if you are sitting like myself next to a laser printer or a Xerox machine. So it is not bad to use something that natural in a process like this.

We tried I would say hundreds of experiments, and I'm just presenting those that seemed to work really the best.

We contaminated eggs externally, we infected them externally. I mean by that is taking warm eggs that has been washed, dipping them in cold *salmonella enteritidis* solution, and let *salmonella* get sucked into the shell. Usually it doesn't pass the membranes, the shell membranes.

Then we take these eggs and subject them to gaseous ozone under a little pressure, ten to fifteen psi for ten minutes, and this is what we got with this experiment. The control was about ten to the sixth. We are inspecting and analyzing the shells only. We separated the shells from the contents, analyzed the shells. The control shells contained about ten to the sixth. After pressure with no ozone somehow we get less recovery. We know that ten, fifteen psi doesn't kill anything, but somehow we got lower recovery, but in the presence of ozone we got nothing on the eggs, which simply means we have eliminated more than five logs of externally-infected eggs.

People are not happy with ten minutes of pressurization. A line of egg processing goes very fast, and they said "Can you do this in one minute?" so we tried externally-contaminated eggs again, in this case we have to combine the ozone with something else, and we tried UV light actually, copying those guys who are using flashes of light, but they had been using white light. We are using UV light, and we can see some reduction due to UV light, but there is a synergistic effect it seems to me between cells that has been exposed to UV light and then exposed later on to ozone.

In this case UV light was done for one minute, and ozone was done for another minute, so a total treatment time of two minutes. This gives the control ten to the sixth; ozone alone for such very short you find about one log reduction. UV alone about two and a half log reduction. The combination about four and a half log reduction.

So one can use such a thing maybe in sanitizing eggs, again taking care of all the external contaminants.

The summary of the results that we have, I probably don't need to go over every piece of information there, but extremely infected eggs we managed to get more than five logs in anywhere from ten to twenty minutes of exposure to ozone gas, and when we have a combination of UV light and ozone gas two-minute treatments produced about 4.3 log reduction.

How this process affects the goals that I set earlier, for aesthetics since we don't dip eggs in any water you probably -- if there are fecal matter on the eggs probably it's going to stay, but we advise that maybe you should wash it first in ozonated water before we do that process that we mentioned.

For freshness, we haven't tested that. Shelf life, we are in the process of testing for that. Safety, we know that we can take care of external contaminants and external infection, *salmonella* that comes through external means, but for internal infection we are still working on it. We are seeing pretty good results that I'm not presenting today.

These are, after four or five years of working with eggs, and after 25 years working with other pathogens I feel that this is the kind of challenges that are facing shell egg safety research right now, how to validate that a new technology is working.

The problem is facility. Can you go just with eggs that are highly-contaminated with *salmonella* and run it in any of these processes and say let us try this, then if eggs break then they have a contamination inside. It becomes a problem.

The other problem is naturally- versus artificially-contaminated eggs. We noticed that inoculation of eggs with *salmonella* doesn't produce exactly the same thing that happens naturally. Naturally-contaminated eggs, they have better distribution of cells in the yolk, and there are many other differences.

I believe also susceptibility of these cells inside the egg is different if you have naturally-contaminated versus artificially-contaminated. But to get naturally-contaminated eggs is very difficult unless you know that the flock is really infected, or if you infect some hens purposely to produce eggs infected with *salmonella* *enteritidis*, a very difficult task.

So if we are going for artificial contaminants what media should we suspend these cells in? Is it a buffer? Is it suspended in egg yolk? What do we do, and what phase of growth do we do. Do we stress these cells before we do that? There are all sorts of questions.

And when you inject this *salmonella enteritidis* into the egg, do you inject it into the white, or do you go all the way to the yolk, and when you inject it into the yolk what rupturing the membrane of the yolk will do to the experiment.

The other issue that also bothers me is disrupting the natural defenses of the egg, and I'll talk about this in a little bit more details. We know that this is approximately how the egg looks like. Forgive my poor drawing here.

And if you look at the shell, this is the first defense that any microorganism getting into the egg has to face, the cuticle, the little thin tiny layer outside the egg. It seems the shell because the shell is very porous, so the cuticle seals the shell and it does provide protection for at least a hundred hours or so. After that I think the protection of the cuticle is gone.

The membrane, shell membranes, there are two of them, even though my drawing says three it should be two.

This functions as a physical barrier to prevent microorganisms, but many microorganisms really can handle this. It's a matter of time and concentration of cells. If you have enough of these cells they will break this membrane and get inside the egg.

After that the albumen, the white has lysozyme which is known to be antimicrobial, it breaks the walls of gram positive bacteria. There may be antibodies coming from vaccination or other means in the white that should provide some protection.

Avidin which combines biotin, biotin is needed for the growth of some microorganisms. If you combine biotin you're probably preventing these microorganisms from growing in the white, other compounds like ovotransferin which binds iron which may be needed by many gram negatives, so that the white is quite hostile to invading microorganisms.

These defenses, they grow weaker and weaker as the egg gets older, but if you weaken any of these defenses during processing of the eggs it may not be a good idea.

The yolk itself is very, very rich in proteins, fats, minerals, vitamins, an ideal medium for growth of microorganisms. Luckily it is that innermost layer of the egg; otherwise would have more problems.

There is a physical barrier around the yolk which is the membrane, but the yolk itself may contain antibodies that is coming through vaccination.

So all these natural defenses, what do we do to these defenses when we process eggs. That is the question I think we should be asking and we should be focusing on.

We know that if we inject *salmonella* in the white and incubate this egg for three days the green ball shows that *salmonella* dies over this period of time, and we inject the *salmonella* in the yolk and incubate it at the same period of time we have growth of *salmonella*. There's no secret about that. So white provides a defense line for the egg.

Other challenges like new practices that is coming, and we need definitely to study these -- continuous washing. If the line shows signs of fecal matter on washed eggs, maybe just divert the line and go back and do another round of washing.

Reminds me with the reworking which has been criticized heavily in other industries, like in the dairy industry and the meat industry, reworking is the cause of many, many problems. Is that reworking causing any problems? Is that the first time around if you didn't wash right you may have *salmonella* getting deeper into the egg, and the second wash would not really do much. We don't know.

So there are potential problems. Also stress adaptation which I'm very, very interested in makes me worry about how much stress we are giving the microorganisms the first round, and if we go the second round are these microorganisms responding at all to that reworking process.

Repackaging which is basically before expiration date take the eggs and we wash them again, I would say this is bad practice but should be studied before I make my judgment.

So in conclusion current practices and new processing technologies for shell eggs should be evaluated against clear goals, and hopefully these practices will allow us to maintain or even benefit from the natural defenses in eggs, and we better also use some new technologies in microbiology to address it to the egg safety that I didn't see much of that research recently.

Stress-adaptive response, sustaining and visualizing techniques, it may provide new answers for old questions that you see in literature all the time, and facility for running egg safety research with similarity tot real world there is a huge need for that, and trying to build one it's very difficult.

Any questions?

Yes, sir. Can you use the microphone, please?

DR. MITCHELL: I was wondering on your ozone treatment what levels, you know, how many ppm of ozone you were using for that.

DR. YOUSEF: We tried also some ozone concentrations, and we ended up with ozone in the gas at more than 10 percent of the gas mixture. That's pretty high.

DR. MITCHELL: It's going to be a few thousand ppm?

DR. YOUSEF: In the gaseous peers. In the water phase we can get 20, 25 parts per million and get about similar results.

DR. MITCHELL: Okay. I didn't mention, my name is Bailey Mitchell, I'm with the Southeast Poultry Research Lab. Thank you.

DR. YOUSEF: Other questions?

[No response.]

MS. SNOWDON: Thank you for summarizing things. I'm Jill Snowdon with the Egg Nutrition Center, and I need some clarification. I'm not sure I was understanding one of your points, and that will lead me to a comment that I want to make sure that it's clear, and that is when you did that nice evaluation and taking a look at the different components and how different technologies impact either the aesthetic qualities or the external or interior safety, and you were talking about washing, the general washing and sanitation practice that's going on in the industry now, were you coming to the conclusion that that was not contributing to external safety aspects?

DR. YOUSEF: Well, I'm saying that washing as far as affecting the natural defenses, it definitely eliminates the outside cuticle, but if the wash water contains high enough sanitizer any dislodged fecal matter will be taken care of before they have a chance to cause internal contamination.

How much that washing process eliminates *salmonella* that got already in the egg by other means, fecal matter sucked into the egg while the egg is being laid, we don't really know the answer to that, and I doubt if it affects that, but microorganisms on the outer surface of the eggs should be taken care of by the high levels of chlorine and the temperature combination, and the high pH is a very, very important factor in eliminating *salmonella* on the outside of the shell.

MS. SNOWDON: That's what I wanted to hear, because we don't want to lose any of the gains that we have gained on public health protection with the washing and sanitation that we're doing in terms of the external, so your concern is there might be something in the shell itself or the interior.

DR. YOUSEF: My concern is the wash water, if I'm trying wash water that doesn't have any sanitizer first I think that's not right, because I may be dissolving fecal matter and getting it into the holes of the egg, and that can be a problem.

MS. SNOWDON: Thank you.

MR. BRACKETT: Are there any other questions for Dr. Yousef?

MS. CURTIS: Pat Curtis, North Carolina State University.

In your schedule or your diagram where you're showing the wash process, was the washing time and temperatures that gave, was that for a single wash system, a double wash system?

DR. YOUSEF: It was for a single wash system using Diamond washer.

MS. CURTIS: Most of the processors now use double wash systems, and there's a little difference in time there. It looked like the rinse temperatures were also a little bit high, but the comment I wanted to make about the wash water, the pH, the wash water is recycled and the pH is mainly to take care of bacteria that would come off of the egg in the recycled wash water, and there's a number of studies that have been conducted regarding wash water, and temperatures, and cold water washing, and a number of those areas that weren't brought out in this that I think are important aspects that we need to consider, because when we look at temperatures of those eggs during that process that's an important concern is how much temperature is being picked up in those eggs during the process.

DR. YOUSEF: What I presented is an example of the wash process. Maybe I shouldn't have said it is a typical wash process.

MR. BRACKETT: Do we have any other questions?

[No response.]

MR. BRACKETT: There was one technology that has been studied a lot in the last year that was not addressed yet, and since we have one of the people who have worked on that I would like to ask Pat Curtis to come back up again and sort of summarize some of the chilling technologies that have been done at North Carolina State.

MS. CURTIS: Actually there's two universities that have worked on rapid cooling of shell eggs, and that's North Carolina State and the University of California, and I'll mention both of those.

North Carolina State has spent a lot of time looking at initial processes from washing to the point of packaging and trying to cool the eggs down, and what we have found is that we went around and did a lot of surveys looking at egg temperatures, and we found that the temperature of the egg during processing rises from twelve to fourteen degrees before we put that egg in the carton, and so it then peaks and rises another five to ten degrees after we package them, put them into pallets, and then either put them into coolers or ship them out.

So you've got an extra little peak there before we actually start any cooling process, and if you actually put a pallet of eggs, thirty cases of thirty dozen eggs in a pallet and in the center of that pallet you measure the length of time it takes that egg to actually cool down to ambient temperature can be anywhere from five to fourteen days, depending upon ambient temperature and air movement and, you know, coolers, and those types of things.

And this is important from the standpoint that we know that *salmonella enteritidis* will grow if the temperature is above 45 degrees there. So both NC State and the University of California have looked at ways to speed up that process of getting the internal temperature of the egg down to 40 to 45 degrees, and what we have done at NC State is we used carbon dioxide as a coolant, and we cool down the eggs, we have a process, we've worked with PraxAir, Incorporated out of Chicago to run eggs through before they are put into the carton, and it takes less than two minutes, and we're getting them down to about 48 degrees, and then they'll continue to cool where that shell was hot and it was going to peak because it was going to continue to heat, at this point the shell is cooler so it's going to continue and in about fifteen minutes after they have been processed they're down to 45 or 41 depending on what your temperature was at that point that you sat.

So that process will be commercialized later this year. It should be at the international show here in Atlanta in January, a regular unit.

The University of California -- and I'll just comment very briefly on this -- has done some research where they're taking and putting them into coolers, and then drawing cold air through the eggs, and it's a little bit slower process, but it is still more rapid than a traditional mechanism. You have to double-stack the eggs because you have to put them into a line and cover them, and then pull the cold air through there, but it does have some potential there of speeding up the cooling of the eggs as well.

So we have worked on the standpoint that if there did happen to be contamination we could control that contamination growth by getting the eggs cooled as fast as possible.

MR. BRACKETT: Thank you. Jill, did you have a question?

MS. SNOWDON: I just wanted a point of clarification, and that is that Humphrey's work at least indicates that SE is not going to grow below 68 degrees for about three to four weeks, so the 45-degree concept I think has to be in that context.

I think I know what you were saying when you said they don't grow below, you know, and I understand the goal there. I'm not arguing that, but I wanted to bring that little detail out that we do have the natural protective mechanisms in the location of the SE in the membrane I think is the current hypothesis in the white next to the yolk.

MR. BRACKETT: And that was Jill Snowdon from the Egg Nutrition Center.

MS. CURTIS: And just one comment on that, and you'll hear Richard Gast a little bit later, but the studies that Richard has done and some of the studies that have been done at Auburn University of inoculated eggs has not shown the same thing that has happened with Humprhey.

We have seen that you have been able to maintain the live *salmonella* within that, and that in some cases it has grown according to some things that we have seen at Auburn.

MS. SNOWDON: The Egg Nutrition Center has a request for proposal out to take a look at it so we can get the data published.

MR. BRACKETT: Any other technical questions that we have for the last speaker?

[No response.]

MR. BRACKETT: Okay. Fortunately, we are a little bit ahead of time, which I think is fine. We will take a break now, and then reconvene back in here at ten-thirty.

Again, we have coffee as well as drinks in the back, as well as donuts and that sort of thing in the back. Please avail yourself to them, and be back here promptly at ten-thirty.

[A brief recess.]

MR. BRACKETT: Okay. It is ten-thirty, if you could begin finding your seats we will get started with the next section.

The next section of information that we are going to receive deals with Objective 7.3, and that really is involving the research to improve testing methodology of SE on the farm and in the eggs, and we would like to stress that the testing that is being looked at is both for individual foods as well as environmental.

This morning to speak about methodology we will have Doug Waltman who is with the Georgia Poultry Lab to discuss some of the methodologies.

STATEMENT OF DOUG WALTMAN, GEORGIA POULTRY LAB

MR. WALTMAN: Thank you.

I appreciate the opportunity to share with y'all an area that is a passion of mine, although my technicians would use the word obsession a little more than passion.

I have been asked to address Objective 7.3 which deals with the research to improve the testing methodologies for SE both in the environment of the farm and in the eggs, and there's five components of this objective dealing with the sampling protocols -- this is the section and collection of the samples themselves, the screening tests or how we detect SE, the development of rapid tests which would greatly help the turn-around time, molecular methods for subtyping which would deal with the epidemiology, and then the identification of virulence factors.

I'm going to specifically address the first four of these, and hopefully Dr. Gast and Dr. Petter will address the virulence factor aspect of this in a following talk.

If we first look at the sampling protocols as they deal with the environment we can look at several programs that have been well established, for example the Pennsylvania Egg Quality Assurance Program. Normally they focus on the manure areas, whether it's pit or scrapers, and we'll talk a little bit more about these housing types, the egg machineries, and these walkway samples.

Now, there is published data from the SE Pilot project which preceded the Pennsylvania program, and they summarized their SE isolations from these various sample sources, and it really didn't make a lot of difference, from fans which was their lowest isolation of SE of about 12 or 13 percent to the walkways which was around 18 percent.

But the way I would like to have seen it analyzed was on a per-house, or a group-of-house basis. For example, if the walkways were all positive out of twenty houses, then you wouldn't need to do any of these other sampling types. But on the other hand if say five of the houses were positive by the walkway, ten by the manure pit, and then another five by egg belt then we would need to do all of these different sampling types, and to my knowledge this data was not analyzed with regard to that procedure.

Another resource that we have is the NAHMS study that is in its final stages. I understand that the final report will be out hopefully this fall, and again they looked at the very similar sampling areas, and I hope that they when they analyze their data that they will do it with respect to particular sources so that we can begin to determine which ones may be more effective than others, and if we can get by with just one.

The FDA trace-back data is another, in my mind a very good resource because it would be on a national level. There's a number of houses that are in this. They have also looked at a few other sampling types, and as I understand it that data has not been analyzed by source, but it would serve to hopefully answer some of these questions about the sample source.

Now, when we talk about sampling layer houses we have a fundamental problem, and that is because of the tremendous diversity of these housing types and the equipment in those, and let me just illustrate this this way: Let's say for example over here we have a house with five or eight thousand birds, here we have one with 80,000, and over there we have one with a quarter of a million birds. Here we have a high-rise deep-pit house, this one we have a shallow pit one-tiered house, we have a manure belt with scraper system here, we have a flush system here. We might have one that is completely environmentally controlled over here, this one doesn't even have walls. This one is hand gathered, whereas this one is completely automated.

So this tremendous diversity can happen even in one state, so when we get to the point where how do we sample these kind of houses the situation is that there's no way presently of developing a standard protocol for sampling, and that is a concern.

For example, the FDA trace-back folks list all of these different housing types, and each of them has its own sampling protocol. And trying to put that on an equivalent basis that we're sampling these houses equally is very difficult at best, so there is a need to come up with a better method to sample these houses, hopefully to put them on the same plane.

Now, there is another problem of major concern at least in my mind, and it's that first one right there, the high-rise deep-pit house, and this could be the most common housing type. This is a situation where the birds are actually on for example the second story, their manure falls down to ground level, the manure domes up, and that's what's called the manure pit, and you have fans and ventilation down there that dries that out, and you have some type of composting that goes on.

But in order to sample that you actually have to get down in that pit and you drag these gauze pads the complete length of the house on top of those domes of manure. Now, if you have never been there that is an experience, I'll call it hazard because it's very dim down there, and to some extent that's not bad because there's things down there that you don't want to know about.

But of primary concern is water accumulation, whether that's from rain, or a leaky drinker, or even the evaporative cooling system that regulates the temperature where the birds are, you can get set up areas that are analogous to quicksand, only this is with manure, and I personally have been in over my knee, and I know of an individual that went in over his head, and it's a very dangerous situation, and there's other hazards down there as well, so from my perspective I'm going after something that I can replace that sample with, and that's my focus, what my focus has been on.

I did a study that was funded through U.S. Poultry and Egg, and that full report is available through Dr. Charlie Beard, and we looked at a variety of different sample sites, even more than what is listed here, trying to determine what would be the best sources either singularly or plural.

What we found was that as other people have shown it's not difficult to find *salmonella* in layer houses. That is fairly common, and you would expect that given the fact that these birds have been in that house, if this is the end- of-lay testing which this is, they've been in there about two years without antibiotic treatment, without a lot of cleaning and disinfection going on because they are food producers.

Now, disconcerting to me from a research standpoint, but good news for the layer industry here in Georgia, we didn't find SE. As extensively as we looked at it we didn't find SE in any of these houses, and so the data that I'm presenting is for generic *salmonella*.

Now, I don't have any reason to believe that *salmonella enteritidis* would respond differently, but I cannot confirm that aspect. And we can see from this that the walkway swab for example detected all of the positive houses as well or better than the manure areas, and just slightly less on a per-sample basis than the manure pit, and certainly both of those were better than the egg machinery swabs and these other dust-type of samples.

If we consider the research needs as I see it we still need to have a valid comparison of SE positive houses to determine what source or sources that we actually need to sample.

If we can get away with just the walkways, or just the egg belts, then we don't need to be sampling these other things. From a cost and labor standpoint that would be very beneficial. Also, from a hazard standpoint it would be nice if we didn't have to get down there in those pits.

A subnote of that is that it would be nice to be able to find a sample that would allow us to put all of these different housing types on the same equivalency, such as it would be nice if the walkway sample panned out.

Along these lines we need to determine the optimum number of samples. There is one program that you can go into a house of 80,000 birds and come out with five samples. Is that enough to tell you the true situation in that house?

And then determine the effectiveness of cooling samples. Most of these studies that I've shown use individual samplings, but I know the California groups have looked into this area of cooling samples, and this would cut down on the number of samples that are tested.

Now, we are going to be switching back and forth between environment and egg, and keep in mind these are totally different situations from a microbiological standpoint. The sampling protocols for eggs are pretty set. It's just the number of eggs that varies. The trace-back when they test eggs it's a thousand eggs, I think the Pennsylvania program depending on the situation is 480, a thousand, or 4,000 eggs.

These are collected usually at random through the house, the environmentally-positive house, the shell is sanitized, aseptically cracked, and the contents, the entire egg contents are pooled into a bag, and twenty eggs to a pool, and we'll show you how these are done shortly.

That's the sampling protocol. If we look now at the screening test, and we keep in mind that a screening test is a very sensitive, usually not a specific test, that then after we screen we then do something in addition to that to actually confirm it, and the question some people ask is "Well, why are you looking for SE in the environment when the egg is what we are concerned about?"

Well, again, this is a screening test. We're using the environment to show the likelihood, or to increase the likelihood of the houses where the eggs might be contaminated.

Now, before I get into the environmental sampling which is what is being done now let me just touch base a little bit on antibody testing, because this is another at least technically feasible way of screening for SE. The problem is it hasn't panned out yet. There are several reasons why antibody testing has not been useful.

One, there's a lot of other *salmonella* in these facilities as was already said, there's a lot of cross-reactivity that goes on with *salmonella* so you can have some specificity problems, but also because a lot of these *salmonella* are not very invasive, or tend to localize very quickly you get a very marginal antibody response in many cases, so not only do you have specificity problems but also sensitivity issues as well.

Now, as part of that NAHMS work they looked at an antibody test I conjunction with that survey, and perhaps they will have some different data to share with us later.

But for the most part everyone is looking at the environment, or environmental culture for the screening test for SE, and this is pretty standard. This is the Pennsylvania program, you add a selective enrichment which is usually tetrathionate, you incubate that overnight, and then you inoculate selective plating media, and then you screen *salmonella*-suspect colonies. That's typical of most programs, for example the FDA group.

Again part of my research project was not only to get some insight into the samples that may be better than others, but also the culture method, the actual way that we can isolate *salmonella*, and we looked at eleven different isolation protocols as you can see here. Again this is generic *salmonella*, we did not find SE, so the data has to be looked at from that viewpoint.

These computer-generated slides didn't like this slanted version, so I'll have to share with you what some of these are.

Notice that there are variations in these different methods as to the percent of *salmonella* they recovered. This procedure right here is similar to what many laboratories are using. At least in our hands this tetrathionate conya is somewhat inhibitory.

The best procedure incorporates a delayed secondary enrichment aspect, but a problem with that is that this procedure takes ten to twelve days, and in most settings that is unacceptable because a quicker turn-around is needed, especially for example in C&D where they need to know now if it's still there so they can do the process again.

So we looked at the possibility of combining a couple of these, and these over here on the left are the singular versions, and then we combined the initial tetrathionate with the delayed, and once again it did produce the highest recovery.

This so-called BAM method incorporates the preenriched tetrathionate with the preenriched rappaport, and this is similar to the LAC-approved method for heavily contaminated raw poultry, and we see that it's in sort of the same ball park, but we can cut out five to seven days with this method over this one, and more than likely this will be the accepted method for culturing these environments.

If we look at specific research needs just to sort of go back over these, as I've pointed out it would be nice to confirm whether or not SE does respond like all these other *salmonellas*. There is a need for rapid detection of SE, and I specifically mean a rapid detection for *salmonella enteritidis*. We don't need a rapid method for *salmonella* because *salmonella* are present on these farms; we need one that will be specific for *salmonella enteritidis*.

Now, that's sort of the challenges that we have to deal with with the layer farm. We've got this huge level of background flora from which relatively speaking *salmonella* is in very low numbers, and even perhaps stressed or sublethally injured, so we've got to pull these few *salmonella* from among the forest, and even then we've got to then screen or identify whether or not they're SE or some of the other two thousand serotypes.

Well, the situation is different with the eggs. We don't have that massive background flora, but we do have several challenges with the eggs as well.

Early work in Britain by Humphrey showed that the vast majority of contaminated eggs were contaminated with very low numbers, less than twenty, or even less than ten organisms per egg.

Now, you add to that the USDA risk assessment said that one in 20,000 eggs are contaminated here in the United States, very few in number. And then several studies have documented that SE contamination is intermittent and sporadic. So you get a situation such as this: You've got a hundred thousand hen layer farm producing 80,000 eggs a day. Today you might get one positive egg, tomorrow none, the next day three, no more for say seven days, you might get one, and that's the kind of situation you have. You don't have a situation where five thousand eggs are being produced each day with *salmonella enteritidis*. I think you begin to start seeing this needle-in-haystack scenario that is developing.

It actually gets worse than that, and Dr. Benson can verify what I'm about to share with you. Remember we're pooling twenty eggs, each egg has roughly 50 mls of egg contents, depending on the size, so after pooling twenty of these you've got a liter of egg material, and you've all broken eggs and you know how viscous and what you have to do in order to homogenize or to mix that up, you've got to beat the daylights out of, so it's very difficult to mix that, get a homogenous mixture, and then you have to incubate that, so we've got fifty of these bags, or jars, or whatever container this liter of eggs is in, and we've got to incubate that, and the standard way is room temperature because there's few labs in this country that have the incubator space for this volume of material. So we do it at room temperature for at least three days.

We then inoculate two selective plating media from this, and again we are going into this liter of material usually with a swab, and then we streak these plates.

So if for example we had one contaminated egg with ten cells in it, we put it in a liter of material, that would have to multiply to roughly around one to ten million organisms in order for us to be able to detect it on these plates, so the old needle-in-a-haystack scenario becomes very probable when you compare it with the situation of trying to find SE in eggs.

Dr. Gast looked at the sensitivity of these procedures. This direct process is what I have been describing, and you can increase the sensitivity of detecting *salmonella* from eggs through enrichment methods, but each time you go through these processes you increase the labor involved, the cost involved, and the turn-around time as well, and with eggs especially we need to be able to get an answer as quickly as possible.

So that's the screening test, or how we're actually detecting the *salmonella* right now, and there is a need as I have already mentioned for these rapid tests, and there are a slew of them on the market. I could use not only my fingers but most of my toes in telling you of all these commercial kits that are already available for detecting *salmonella*.

The problem is that they were developed for food and food products, and they have the LAC approval, and they work very well with that setting and in that situation, but the environment of layer houses are entirely different.

The Arkansas group looked at three, the reveal, the bind, and a filter method, and their conclusion was that they did not recommend these rapid detection methods in their present state of development.

This group looked at a genus-specific PCR, and we don't even have to look at the results of their research to tell you that it's not going to help us, because remember we said that *salmonella* is present in these layer houses, so a yes-no test is not beneficial because we've still got to culture it and then determine whether or not it's *enteritidis* or not, so this is not of any use for us.

We also looked at six different kits. This is the best isolation here culturally, this is the BAM from which all of these are sort of evaluated against, and you see that they performed comparable to the BAM, but again all this is telling us is yes-no *salmonella*, and it really doesn't help us because of the level of *salmonella* that's there.

In a food where .1 percent of the samples may be positive for *salmonella*, rapid kits are very effective because they screen out the negatives, you only culture the positives, but in a situation in a layer house where 50, 75, maybe even a higher percentage of samples are positive these rapid kits that are generic do not help us.

So the research needs, we do need the rapid kits, we need to increase our turn-around time, but they must be specific for *salmonella enteritidis*.

With eggs there has been some work looking at kits from the antigen capture elisa formats, as well as PCR, but again just because of the matrix that eggs, those massive pools, all of these require some type of enrichment, and even to antigegen capture elisa, most of these require a level or around ten to the four or higher in order to detect them, so when we talk about rapid it's not in the sense that we normally think of rapid. We may be cutting out a day or so with these, but again they are *salmonella* specific.

And then the final component is molecular methods for subtyping SE. Again, *salmonella* is a huge group of organisms antigenically. There's well over two thousand serotypes of *salmonella*, of which *salmonella enteritidis* is one. We already have one method of dividing SE, and that's the phage type, and you've heard phage type 4, phage type 8, et cetera.

But within those we don't have, or it would be nice to have a method of dividing those isolates out for epidemiological purposes. For example, if we had forty isolates of phage type 4 it would be nice to know if they were all clonal or if they were of diverse origin.

So various methods have been used, plasmic profiling, ribotyping, pulse field gelalit, electrophoresis, and random amplified polymorphic DNA typing, or rapid.

All of these have been shown to work in various laboratories to be able to discriminate between isolates of SE. One of the problems, though, is that if I have forty isolates of SE and I do ribotyping on them for example I may get eight different groups, and if I do the rapid procedure I may get 25 groups, and this group and this group don't have any relationship at all, so then it's almost like apples and oranges how I compare this, and certainly when different investigators try to compare their results with one another it's a confusing mess.

So it's not that they don't work. What we need is the acceptance of some kind of standard. Whatever we choose, if we standardize it then we can start comparing the results from various locations, from various laboratories, and start making some broader epidemiological statements, and it perhaps would be beneficial to have one laboratory that's doing this testing. That way you don't have reproducability problems, you don't have differences in perhaps the way the method is being done; you have a central repository that is looking at this data.

So what I have tried to share with you briefly -- it's not really briefly I guess -- is the current status of our detection, our monitoring and detection program, and to also try to share with you at least from my opinion of what some of these research needs may be.

And if there's time I'll take any questions.

MR. BRACKETT: Thank you.

Yes, Peter.

DR. HOLT: Peter Holt, USDA, Southeast Poultry in Athens.

Could you go over the BAM technique that you talked about which seems to be the accepted procedure?

MR. WALTMAN: Well, again, the BAM, this is an FDA protocol, it's what is used for testing food and food products. It's the accepted standard for example that everything else is judged against these rapid kits or whatnot.

And depending on the food type, that method may be different, but for example for heavily-contaminated raw poultry the procedure that is recommended is preenrichment followed by tetrathionate and rappaport baccilioitis. Okay. You preenrich the sample, and then you inoculate both tetrathionate and rappaport, and then you go through the plating and the processing from there. It's a dual enrichment procedure.

MR. GODFREY: David Godfrey, Georgia Tech Research Institute.

Has there been any interest or any studies of airborne sampling for either generic or *salmonella enteritidis* specific?

MR. WALTMAN: Well, in layer houses there has sort of been word of mouth detection. It has been shown that SE is airborne transmitted, the group Peter was mentioning, or Bailey was mentioning where you can infect birds by an airborne route. I don't know that anyone has shown that in a field situation. I have talked with the CDC individuals, and they know of no situation where for example a worker in a layer house has been infected with SE by that route.

MR. BRACKETT: Thank you, Doug.

As you can see, we're moving from the more practical into the much more theoretical, and in some cases much more difficult questions.

The final research objective actually dealt with more of the fundamental questions that affect all of the other previous ones, and that is Objective 7.4 which is to conduct research to understand the ecology and the epidemiology of *salmonella enteritidis* in the hen and farm environment.

Again, we will have two individuals from the Agricultural Research Service to talk about that. First is Richard Gast who will discuss more of the ecological aspects.

STATEMENT OF RICHARD GAST, ARS, USDA

DR. GAST: Good morning.

Actually as Bob alluded to it is sort of interesting in approaching these objectives in the order that they're published we're ending up possibly at this point in the program having what might turn out to be sort of an introduction, because much of what I'm going to do is really along the lines of an overview.

I'm a little bit raspy this morning. I was commenting to my colleagues on the way over here, having already passed out copies of my slides to all of you we could just turn the lights on and do this like and eighth grade social studies class and go around and have each of you read one of them out loud.

Just a stray thought, and one that I suppose will be disposed of immediately.

Over the past few years as most of your quality assurance and risk reduction programs have been developed, if we look at these we can see that these programs tend to involve what I guess we might call the broad spectrum strategy of approaching the problem of controlling SE in eggs by applying a coordinated series of responses across the entire continuum that goes from breeder flocks, to egg-laying flocks, on to the processing, storage, and preparation of eggs.

And although it has been argued with I think considerable effectiveness and considerable merit that the post-production arena is an area in which there are many particularly cost-effective responses available to us, nonetheless historically and still at the current time the laying house remains one of the primary battlegrounds for our war on SE.

Accordingly, an assortment of questions, understanding how SE gets into the laying house environment in the first place, how it survives there, where it survives there, the nature of the interplay between the pathogen, the laying house environment and the biology of the chicken that ends up resulting in the production of contaminated eggs, all these kinds of questions are important pieces of the SE control puzzle.

It's interesting looking at the title of this this morning, all of these things in some vague way, all of these kinds of questions are what we tend to end up referring to as the epidemiology and ecology of SE.

To be truthful, I think most of us realize these words are pulled in from other disciplines, they're not probably exactly precise or applicable to this situation, but we do have a general sense of what we're talking about, and at least this provides us a common vocabulary.

What we're really talking about here is put in simplest terms what goes on in the laying house, and how it results in birds becoming infected and contaminated eggs being produced.

What I'm going to try to do this morning is review in a very superficial way some of what I see as the principle issues related to this topic, and then provide some personal opinions about what I think are valuable research areas that are worth pursuing.

It's kind of interesting looking around the audience here, I'm probably not the most appropriate person to give this talk. It's interesting to me looking and seeing people like Andy Rohr and Eric Ebel that were in the trenches quite literally, and unfortunately for them, years ago dealing with this situation, Marilyn Baumer who's dealing with it today, people like Chuck Benson who is probably arguably the father of research on SE in the U.S. -- if his beard gets any longer we'll have to start calling him the grandfather. You don't get the microphone until later, Chuck.

But there are a lot of the rest of you that can and should contribute to this, and I'm somewhat of an outsider to this issue of what goes on in the laying house. I'm a research laboratory guy, but I hope that the perspective I provide at least as a laboratory researcher and as a student of the literature may have some bearing.

I think the principle issues on this topic can really be grouped into three broad categories, first those things that relate to the course of infection in individual birds and how contaminated eggs end up being produced by infected hens; secondly, what the sources of SE are, and; third, the reservoirs that enable the organism to persist, and the mechanisms by which it is transmitted between birds within houses.

I'm going to divide these up into three broad questions and try to look at a little bit of what we may know and what we think we need to know according to those three categories.

First, how does SE infection of laying hens result in the production of contaminated eggs. Much of what we know about SE infections doesn't different them a lot from other paratyphoid infections of poultry. They establish intestinal colonization quite effectively.

This is an experimental infection study from some years ago where we gave laying hens very large oral doses of SE, and you can see we established -- these results are from the period during the first month after we infected the birds -- colonized the intestinal tract quite nicely, spread to internal tissues including the liver and spleen, and of greatest significance for egg contamination, also makes its way to reproductive tissues such as the ovary and the oviduct.

It's also interesting if you look at the birds that are listed here as contact exposed, those are birds that were not orally infected, but simply placed in cages in the same room with the infected birds. You see relatively similar results. This organism is horizontally transmissible, and those perhaps provide us a little bit better model of a natural infection.

As you would expect from the fact it gets into reproductive tissues it also of course makes its way into eggs. It's distinctive about SE that unlike other *salmonella* serotypes which are at a fairly reasonable frequency historically known to be deposited on the shells of eggs, largely because eggs and feces exit the bird via the same opening, SE is also at a considerably elevated frequency compared to the other serotypes found in the contents of eggs.

We can see this again from an experimental infection study where we sampled shells, yolks, and albumens and found it in all three. Actually if you look at second week there we're finding it in yolks and albumens at frequencies higher than we're finding it in shells, suggesting that internal contamination doesn't seem to be very strongly related to external contamination.

Some good work that was done at the University of Pennsylvania a few years ago corroborated that by finding SE in the developing contents of eggs before the shell was even secreted around it.

A couple things about this that I think you have to keep in mind that are distinctive and in regard to the real-world situation, remember these are experimentally infected hens given giant doses of SE, so there are some things here that are unrealistic.

First of all, the responses are exaggerated. You will never find these kind of responses in naturally-infected, in eggs from naturally-infected birds. The risk assessment data from USDA from several years ago suggested that a one-in-twenty-thousand kind of incidence nationally is more realistic.

Also the kinetics of this are pretty artificial as well. We see all the birds infected at the same time, they're all producing contaminated eggs, or they're producing contaminated eggs predominately for about a two- to a two-and-a-half-week period after infection, then it drops off.

In the field of course every bird isn't going to be exposed on the same day, it's going to roll through the flock, so you won't see that same kind of trend, but you would expect a wider distribution over time.

Interestingly, though, in the field there still tend to be little clusters over time where something is going on that seems to trigger a little burst of egg contamination, whether that means a new group of susceptible birds are being infected at that time, or some management factor has changed susceptibility to the infection and allowed contaminated eggs to be produced.

I should note just a -- actually I can go back -- just a real footnote, and this is an area that that was alluded to earlier, and I don't have time within this presentation to get to, note that in our experimental infection study history both -- and this is an old study, and we have repeated these things several times -- even in very recent studies we see both yolk and albumen being contaminated experimentally at comparable frequencies.

Now, when we see yolk contaminated it's almost invariably associated with external structures of the yolk, the membrane. It rarely seems to be in the contents of the developing eggs. But this is somewhat in distinction to what Tom Humphrey has reported in England in that whole issue of is it in the albumen where it's unlikely to grow, or is it near the yolk where it's more likely to grow is I think not quite as simple as it may be portrayed on the basis of Tom's research.

I don't know if either his lab research or my lab research accurately reflects the reality. I think this is a black box area. It's not part of this presentation, but this issue of where is SE being deposited in eggs is critical to understanding what might happen subsequently during refrigeration.

An interesting characteristic we've seen consistently of SE infections, they are pretty persistent. When we infected as Pete mentioned earlier day-old chicks with moderate doses, around ten to the sixth, you could see that although it was cleared out of internal tissues after the first month post-inoculation it stayed in the intestinal tract for quite some time, even considerably longer than this graph shows. Actually at 24 weeks of age most of these birds had grown -- by that point they were laying hens and were still infected, more than half were still carrying the organism in the intestinal tract at that point. Some of those birds also laid contaminated egg infected as day-old chicks.

Also you can sometimes see very considerable persistence in laying hens, although usually not quite at the same frequency.

Finally, as Doug was alluding to, the antibody response of birds is a characteristic of infection that we keep coming back to wondering what to do with it. Experimentally-infected birds, again given large doses, do produce very large antibody titers easily detected for a long time, six months or more post-infection.

It's very tantalizing to believe that those are out there. The same thing for the contact-exposed birds, by the way, although you can see the elovars, it takes longer for the response to develop, but again significant titers, long duration. It's a very attractive target, but as Doug alluded there are a lot of other factors that influence whether that response is a meaningful target for protection.

Some of the things that I think that are still worth doing, that we still need to know in regard to this first category of SE infections, I think we need to know still more about bacteriological and serological characteristics of hens that are useful for detection.

When we think about detection we're often focusing on the technology itself of finding new primers in PCR tests, things that are related to the details of the test, but we also need to know the details of the infection, what does the bird, what antigens does the bird express, where is the organism found in the bird, and so on, so that we know what to go looking for. This is particularly I think a consequence in the antibody tests.

Secondly, the same sorts of information, details about the infection, how the host responds to it, so that we can look at some of those intervention strategies.

When we think about vaccination the same thing applies. We need to understand how the infection proceeds in the bird, and how the bird responds to natural infection so that we're better able to develop strategies that circumvent that process and prevent infection from leading to contaminated eggs, or, better yet, preventing infection from happening in the first place. But some of that depends on better understanding the infection process itself.

Third, and this is one I guess of more personal interest to me as I alluded to a few minutes ago, better understanding of how SE is deposited in eggs -- where, when, how, what kind of numbers, because that's terribly relevant for understanding all of those post-production intervention strategies such as refrigeration and what effect they're going to have.

The second broad question, how is SE introduced into laying flocks in the first place. An assortment of potential sources, all of which we have discussed many times over the years, the first one that always comes to mind since the organism we know is deposited in eggs it's not surprising to us that it's also vertically transmissible from parent to offspring, and even worse is the fact -- and there's been a nice body of research done by my colleagues at the Russell Research Center in regard to broiler chickens that hatchers are places where there is immense opportunity for rapid and prolific spread of *salmonella*.

You have birds at the most susceptible point in their entire life crowded together with rapid air circulation, lots of dust and moisture circulating around, it's an excellent opportunity if it was there in the egg, and if any of the material in there is contaminated for a large number of birds to become very quickly contaminated.

However, let's also keep in mind, although this is an immensely important potential source, this is one of the areas in which we know probably both the most information about what's actually there, and have probably arguably the best control program already in place, and both of those, the information and control program are in the guise of the National Poultry Improvement Plan which specifically targets this issue, so we've got a good data base of what's out there, we have constant ongoing monitoring, and we know to what extent I think fairly precisely that this contribution at least from the chick standpoint is leading into SE, or leading the SE problem in laying flocks.

Second, the poultry house environment from previous flocks looks to be a major player in the ongoing problem. There was a very good Dutch study some years ago that looked at when flocks became positive over time, and a very, very significant percentage of them first became positive after transfer and placement into the laying house. And I think there's an increasing emerging consensus that more of the battle, more of the issue that we're dealing with today has to do either with flocks becoming infected because they're put into laying houses that were contaminated previously, or there's some other environmental source introducing into the laying house.

Third, all kinds of things -- I mean every invertebrate and vertebrate that we know of seemingly can carry *salmonella* either on its legs, or inside itself, and so on, and it's so easy to get trapped into one-dimensional thinking about the flow of *salmonella*, chickens to eggs to humans in our human arrogance. Because we're the ones that we care about most getting sick we tend to forget that we are also just an intermediate arrow in some other pathway.

Human workers can bring it back into laying houses, humans can transmit it to each other, and so on. We have a very complex picture of the transmission of *salmonella* amongst all of the potential hosts, including poultry.

Finally, feed is always a potential source. Certainly we know that many feedstuffs, especially those that contain animal products, are potentially contaminated. Feed sampling almost invariably has failed everywhere in the world to show significant levels of SE. It's hard to pin down feed as a source, but feed sampling is another of those needle-in-a-haystack situations. You get a little cluster, a little bolus of contamination somewhere in there, you know, two grams of it in a silo that might be responsible for a problem, but even though everybody agrees that feed is a potential source it's almost been impossible to really identify contaminated feeds.

The Pennsylvania Pilot Project some years ago is still at this point until we have the NAHMS data probably our best available field study that relates to some of these sources issues.

Some of the principal things we learned from that include the fact that first environmental samples, or the presence of the organism in the environment is indeed relevant to whether it ends up in eggs. Looking for it in the environment correlated very strongly with whether it showed up in eggs.

Secondly, above and beyond every other thing that they looked at mouse infestation in houses looked like a major issue. Heavy mouse infestations were very, very consistently associated with a higher likelihood of the environment being contaminated. Lots of nice connections have been shown subsequently by David Hensly and other people in Pennsylvania between the organisms found in these mice and the organisms that showed up in the flocks, and subsequently in eggs, and so on.

And third, one that's kind of ominous for us when we start looking at what we're actually achieving in the laying house, in the Pennsylvania study only 50 percent of the time was cleaning and disinfection effective in cleaning up a contaminated environment. That's extremely critical if we think of that issue the Dutch are arguing that it's the laying house environment that's introducing it to subsequent flocks anyway.

Where do I think can we go with this in the future? First of all, I think we need to continue looking for what the prevalence of SE really is in all those potential sources -- breeders, chicks, rodents, insects, feed, environment after C&D and so on. That's a little bit different from a lot of questions we're asking.

We have been, logically I think, often very interested in asking where can we find SE most efficiently in order to detect infected flocks, so a lot of our questions have been related really to sampling methodology. We wanted to know the best sources in order to identify an infected flock.

That's not the same as identifying which of those environmental sources are in fact the ones bringing it into the flock in the first place, and some attention to looking at those sources and where it is I think may help us understand where it's coming from.

An epidemiological approach secondly, looking for the relationship between the isolates in the different sources, looking at the input sources and the output in terms of eggs, chickens, and/or eggs is important.

We still this far into the game are struggling to find good epidemiological markers that will really distinguish which sources matter. That's I think a really critical point of issue.

Third, some geographic questions and some management questions I think are relevant, because as Doug alluded to a few minutes ago there's considerable diversity of what's going on out there, and it would be nice to know if the kinds of sources that are involved are in fact the same say in California as they are in Pennsylvania, or Ohio, or Indiana, or Georgia, or any place else, and in addition whether they're the same in these very drastically different types of management systems.

Fourth, we do need to know the effects potentially of all the kinds of intervention strategies that we might apply in the laying houses -- C&D, testing plans, rodent control, feed treatments, and so on, on the sources of SE and on the resultant possibility of egg contamination.

The third question is how does SE infection spread within flocks once it gets there. We've got an assortment of potential natural routes that we know of by which birds might become infected.

I mentioned vertical transmission before. The classic mode of *salmonella* infection is via oral ingestion of organisms from all kinds of sources.

Third, inhalation of either aerosols or dust particles certainly seems increasingly like a possibility, not only because inhalation might lead to respiratory infection, but inhalation in the case of a bird because the nasopharyngeal connection there might be simply another way, or an effective means of ultimately infection via the upper part of the gastrointestinal system as well.

And fourth there are some Japanese folks who have put a lot of emphasis on this one, at least in an experimental context -- I don't know how relevant it is in the field -- is ascending infection up the other direction, either through the gastrointestinal tract or up into the reproductive tract. That works very nicely in the laboratory; I don't if that's any kind of a real world thing or not.

But considering those ways of infecting a chicken you've got an assortment of means it can be transmitted around in laying flocks. Direct bird-to-bird contact of course is a major issue; all kinds of factors, many of the ones I mentioned earlier, both biological ones that are infected themselves, the mechanical ones that just carry it around.

Insects are certainly commonly shown to be carriers of SE in poultry facilities. Mice look like the principal target, though, I think for the most part.

All the things that we might call fomites for lack of a better word, basically every physical thing in there, all the surfaces and equipment in the house can certainly be physical sources of transmission.

And finally one that's been of interest to us in our laboratory as Bailey was showing you earlier, air circulation.

We're going to get a sense of how transmissible these things are horizontally. When we did an experiment a little while ago where we infected two birds in a group of twelve -- these were relatively young chicks, and they were given very small doses, the two that were infected were only given a dose of ten to the third, and then we monitored the other birds after about a week, and with an assortment of isolates the vast majority of the other birds eventually became infected as well. This organism, if the birds are in close enough quarters it's very, very transmissible.

Interestingly enough, this is just a total aside point on here, it's interesting to us that the phage Type 4s that we were so terribly worried about seemed to be a little less transmissible than some of the other phage types. I don't know if that's a meaningful comment or not.

Bailey told you about some transmission cabinet studies that we did in which he was looking at the effect of the ionizers, but in some earlier studies we did just looking at the possibility of airborne transmission we saw that when we infected upstream birds and then looked in the downstream cabinets when we did surface rinses of the exterior feathers from those birds in the downstream cabinets 77 percent of them ended up being contaminated.

Remember, these are birds that there's no possibility of contact. The only contact between the groups is air flow. A third of those birds ended up carrying it in the intestinal tract, and a fair percentage of them ended up having it in internal organs.

I don't know if that 11 percent that showed up in the lungs if that's inhalation into the lungs, or the lungs may also be an end point tissue like spleens. That may be the result of oral ingestion followed by systemic dissemination to tissues including the lungs. I'm not offering this as some sort of defense of inhalation respiratory infection.

In fact, I tend to think, my own gut reaction here is more that the surface contamination is probably resulting from a lot of oral ingestion; hence, the higher level of cecal contamination. Nonetheless, airborne movement is clearly I think a very relevant mechanism for dissemination.

Some of the needs I think for further work in the transmission area include determining the prevalence associated with the various transmission mechanisms -- dust, moisture, rodents, insects -- I think those are all relevant. We should add moisture as an issue. Colleagues at the University of Maryland have been very interested for some years in moisture and water activity levels as a means of perpetuating *salmonella* survival in broiler houses, and I would presume that many of those same issues apply in laying houses as well.

In fact, any of you who are on Ed Malinson's mailing list, Ed has been very evangelical in making the point that controlling water levels, moisture levels in poultry houses is a very affordable technology, it doesn't require significant rethinking of how we're managing our flocks, and if it's indeed relevant to the *salmonella* levels it offers us an opportunity for progress without completely technologically changing what we're doing.

I say determine the prevalence associated with the mechanisms here because realistically in field studies it's going to be very hard to document which mechanisms are actually responsible for transmission, but all we can do is look at the potential target mechanisms and try to see which of them seem to be identifiable as places where the contamination is.

Secondly, looking at how current laying house management practices are affecting these kinds of things, affecting the sources and transmission.

And third, I think looking at potential intervention strategies for disrupting transmission, rodent control, dust control by the kinds of things that Bailey is looking at, moisture control, I think those are the three things that come to mind most quickly.

A quick summary here -- this is actually just sort of the general theme of what I've been saying all along -- I think that research to better characterize and understand all these kinds of things going on in the laying house can indeed directly help us come up with some usable tools.

However, as much as I do believe this I think that coming up with some better tools that can help us deal with SE infections in laying houses is a really worthy goal.

We need to remember, nevertheless I think, that what goes on in the laying house is still only a small part of I think our overall targets for how we want to try to control SE.

I think that that broad spectrum strategy is still our best overall option for having long-term success in reducing the problem.

If you look at this realistically, if we look at both the technologies we have available to us, or are proposed in ideas we have for research here, and we look at the technologies available to us for managing egg production, for controlling pathogens in egg-producing flocks, completely eliminating *salmonella* or any other food-borne pathogen from egg production flocks anywhere in the very near future is not I think an attainable or a reasonably-attainable objective, and so I think we need to think of these things very specifically and only as a component in that broader spectrum strategy.

What I'm going to do now is introduce my colleague Jean Guard-Petter who is going to take this I think to a level of a little bit more intense focus and look at some very specific issues related to epidemiology and ecology questions.

STATEMENT OF JEAN GUARD-PETTER, ARS, USDA

DR. GUARD-PETTER: Thank you for having me here today, and what I think we're all realizing as time goes by is just how integrated the chicken, the egg, and the environment is, and unfortunately with SE I think the devil is in the details in understanding specific control measures, specific things that we can do to reduce the current problem. The topics in microbial pathogenesis with special relevance to egg contamination that I work with are outer membrane complex carbohydrates, primarily a molecule you've heard me speak about before called lipopolysacrite.

I worked on a process that some bacteria can go through called high cell density growth, and for those of you who aren't familiar with this, this involves cell-cell communication between bacterial cells through chemicals that they release in the environment when they're growing.

So this is one thing that we know that *enteritidis* can do that typhimurium so far has not been demonstrated for typhimurium, but *enteritidis* definitely can grow to high cell densities.

Whether or not it's doing it in the classical method that relies on a certain thing called the ACL-homoserinlactum [ph] we don't know yet, but I am collaborating with people at Iowa State, Peter Greenburg, to answer that question.

Finally I also studied proteotomes, and this is the changes that occur in protein expression that appear on the surface of the bacteria in response to environmental stimuli, so you can have a major change in proteotome without having a change in the genetics of the organism, because what you're doing is you're entering different modes of gene expression, so this has special relevance to vaccine development.

So proteotomes have a lot of relevance to vaccine development. The high-cell density growth work has a lot of relevance to the development of science-based regulations for better control, and finally also for improved epidemiological monitoring we have proposed analysis of lipopolysacrite structures as a method of subtyping SE.

Now, I just want to show you -- this is using a genetic approach to analyze the contribution that different proteins make to virulence in birds, and it's not that anything we have done here is too different from what's been done from typhimurium. In fact, I rely heavily on the immense amount of work that's been done with typhimurium.

But here we're asking a very specific question, we're asking what is the relative contribution of flagella in this case to virulence of SE. Now, this has been studied in a number of different ways, but what had not yet been done was incorporation of new information that when you have flagella genes in different classes, and there are three classes of flagellin genes that are required to interact with each other to wind up with the molecule for motility, if you have a mutation in the Class 1 flagellin master operai, it actually is integrated then into other regulatory circuits in the cell, and so people had not asked specifically what if you mutate a Class 1 gene, and then compare it to a Class 3 gene, which is the structural gene for flagellin.

And what it turns out is that when you mutate a Class 1 gene, here it's fldD, what we found was a hundred fold increase in oral invasiveness of the organism, and that has not yet been reported.

So what we're finding is that flagellation which is a major out-of-membrane marker on *salmonella*, on all *salmonella* except the avian-adaptive ones, flagellation is not contributing to oral invasion, it's absolutely required for what happens afterwards, so once the organism has gotten into the bird it appears to be directly linked into the ability to grow high-cell density.

And a way, another way that we know that these are two separate compartments of virulence, in other words issues involving oral invasion may be quite different from what you need for control of something that has already gained access in the bird and is now growing like crazy.

We also took a look at SipD. Now, SipD is a proT that is a *salmonella* invasion proT, and in typhimurium they know it's involved in virulence, they have a whole slew of these *salmonella* invasion proTs. They have investigated extensively, and this is a lot of Jorge Galon's work.

Well, again we go to *enteritidis* and we find a slightly different picture. Yes, SipD is absolutely required for oral invasion, and if you knock it out you won't get any *salmonella* in the birds, but if that organism has some way of gaining parenteral or internal access to the animal -- in this case we just inject it -- what we find is that the SipD mutant is not attenuated at all. In fact, it grew in organs a little bit better even than our wildtype strain did.

So we're starting to see ten- and hundred-fold differences between the oral invasion compartment and what happens afterwards.

Now, *enteritidis* as far as we can tell differs in only one major way from typhimurium in regards to oral invasion, and that has to do with another class of genes that we're working up, and it's called the glucoseal transferasis. Again, the devil is in the details with SE, so I'm not going to go into that, but just to let you know we're now investigating very particularly how typhimurium differs from *enteritidis* which has a different epidemiological pattern.

So I mentioned that we worked with lipopolysacrite, and I'm not even going to show the structure, the detailed structure to this group. I'm just going to show you what we're doing with it, and mainly we're concerned about how this molecule contributes to the virulence of *enteritidis*, but not to typhimurium's virulence, because we now know typhimurium doesn't make it. Only *enteritidis* makes this particular form of what's called lypopoly high molecular weight, lypopolysacrite.

Now, there is another important organism that also makes high molecular weight lypopolysacrite, and that's *salmonella typhi*, so we know at certain times as *enteritidis* is going through these bouts of infection and depending on where it is in the bird that it's actually converting and looking more like *salmonella typhi* at times, so what is the role for high molecular weight LPS, and what we're seeing is mitigation of clinical signs in hens of active infection.

Now, here's what we did. We took wildtype SE and a mutant of SE that cannot make high molecular weight LPS, but in all other aspects is a highly virulent strain, and we challenged some hens, and we did use an intravenous route because we wanted to produce a cluster of contaminated eggs.

And what we found was that at this dose -- and there is some dose specificity to doing this sort of experiment -- at this dose both groups of birds produced about 10 percent contaminated eggs in the size of cluster of eggs that we collected here.

Now, one interesting thing popped up about these eggs. If the organism could not make high molecular weight LPS we saw a huge peak of soft-shell eggs that correlated with egg contamination. If it was making high molecular weight LPS, the shell remained in good shape as far as we could tell, because within my little lab we don't have anything fancier for judging eggshell quality than our technicians' subjective assessment.

So here's what we found was that about 39 percent of the eggs following the day one of challenge from those receiving the mutant that can't make high molecular weight LPS were soft, and it was so obvious they were soft, some of them were like lizard eggs, some of them just smashed as they were collected, and so what we're wondering is you see a tiny, tiny little bump here. Now, that may or may not mean anything at all, but what if strains that are making high molecular weight LPS are sub-clinically altering shell quality just enough that maybe we could use improved technology on a high throughput basis to assess egg shell quality.

Now, this is a different sort of correlation with a change in shell quality than the classic we cracked the egg shell and the organism got in. This is a change in egg shell quality that comes about from the bird having picked up an infection.

Now, you'll see here that we get another little cluster of soft shell eggs. In this experimental model what is happening is these birds are suddenly beginning to increase egg production, and so that may be an artifact of the experiment to model, but what we definitely see is at least an uncoupling of contamination with the change in egg shell quality if the bacteria can make that special form of LPS that I've talked about before.

Now, the other thing we know is that the egg is a selective environment for strains of SE that produce typhi-like LPS, and let me tell you how we determine this.

We do a lot of chemical determination of serotype, not immunological. We are not interested in that one little sugar that determines Group B or Group D; we look at all the other sugars, which there are a lot more sugars on that LPS, so what we're doing here is we're plotting the amount of rhamnose against the amount of glucose in LPS.

Now, typhimurium produces an average LPS structure that clusters right here where you actually see a cluster of structures from SE. Now, all of these data points represent a different SE isolate, and so here we see a nidus or a focus of structure, and from that structure then there is a diaspora of structures coming out of it.

Now, this is a quadrant analysis where we're actually correlating where the LPS structure falls with the virulence outcome in birds.

Now, this typhi structure here has been the one associated most with high levels of egg contamination in our experimental animal challenge model, and what we have found, though, is that if you take an egg isolate and store it for more than a year it starts losing a lot of these sugars on the LPS, and it will fall down into this range. But all you've got to do is pass it back through the bird and the egg will eventually select back out then for the typhi form.

Why is that? Well, the typhi-like LPS that we deal with acts as a capsule for this bacteria. I think everybody here is pretty familiar with, or most everybody here is familiar with the fact that capsules always impart some sort of survival advantage to SE.

Now, remember what I said, SE makes this, typhimurium does not. So we have this now molecular marker for strains that have particular ability to do this.

Now, we actually now know that we can manipulate this glucosilation -- as you can see here it's glucose on this Y axis -- we can manipulate glucosilation by the growth conditions by letting, by giving some stresses and it will pop up, and most of these have to do with something that happens in the egg, which is the egg has a very basic pH, the white does, so we apply stresses that are the same as either oxidative stress, or alkaline stress. They both induce the same set of genes that kind of overlap and intermingle, and so we have a feel now for what it is in the egg that might be contributing to the problem.

We know that what winds up is that the molecule on the surface changes to resemble something that is associated with human adaptation of *salmonella* to people, because as you know typhi is adapted to the human population.

Now, all of these little red marks here, these all came from mouse organs, mouse spleen. The squares came from chicken organs. We don't see the chicken organ isolates popping up into the high glucose range. The mouse spleen is the richest source of LPS structural diversity I've ever seen.

Whereas the egg we know we've got a good fifty-fifty chance of recovering the typhi molecule, the organs of chickens we know it's probably just going to be the average typhimurium structure, the mouse is all over the board. It is spreading all sorts, forms of isolates out there in the hen house.

So one of the research needs I can visualize is someone actually studying mouse populations specifically following the -- as much as we do say of chicken population.

It could be there are some dynamics of *salmonella* infections in mice that are escaping our detection methods in the way that we view mice right now. Nobody is doing epidemiology in mice. However, I do think there's going to be some ways to do it.

So anyway, some applied research needs. I think that we can modify some existing equipment, some existing technology to assess shell quality, and it has been suggested to me that laser air puff technology would be appropriate.

Now, this is amazing technology, and it would be for assessing shell quality. A huge flat of eggs could come through, this tiny little jet stream of air under high pressure comes out, and it puts a dimple where it hits the egg.

Now, a good shell should barely be affected at all, but the laser comes along and measures this dimple. Okay. So you get a readout, a digital readout, and this could be very high throughput, you know, thousands of flats coming through here at a time, and then you see a flat coming through perhaps from a flock or a farm, and if all of a sudden that baseline pops up then perhaps maybe we're encountering one of those clusters of contaminated eggs, and I think it would take something like laser air puff technology to detect this sort of change, because the bacterium has figured out a way to fool our eyes, or to fool the grading people who are sitting there, and I'll say these are putative correlations on in-animal models, it is something we've seen now on three different experiments. We weren't able to get a handle on it until we got into the genetics and had our mutants that allowed us to make these correlations tighter.

But anyway, I do think that this laser air puff equipment technology is promising, it is patented by the Center for Food Safety Quality and Enhancement down in Tifton, Georgia -- is it Tifton or Griffin?

VOICE: Griffin.

DR. GUARD-PETTER: -- Griffin, Georgia, and Yin Con Hung has been my contact there, and I would love to work with him on this, but there are some barriers to us working that we'll have to address.

Now, with my experimental hen challenge model I can begin to look at parameters that might alter or maximize egg shell quality differences.

Now, this might be important because what if a farmer feeding his birds one way has more eggs that sneak through the grading system than say a farmer who feeds his birds another way, so I think there is a need to look at some of the nutritional effects on egg shell quality following a hard challenge, even though it's in an experimental system.

And finally I think with very little risk or downside it would actually be possible to take powerful technology like this, go ahead and take market eggs and start establishing a parameter, a data base, a base line about what is the quality of the egg shell at market.

You could even think of experiments where you select out a bunch of eggs that seem to have poor shell quality compared to others, and culture those, and see if the rate of contamination is higher than the background set say by the risk assessment data base, or the approach, where are we getting back more than one in 20,000 eggs from those eggs with poorer shell quality, because remember these are eggs that probably passed through grading, but perhaps fooled the eye of the grader.

Now, I just want to reiterate, and I think in terms of how human problems and animal challenge models relate, I do think the typhoid fever model has particular applicability to the SE problem, as much as does the gastrointestinal diarrheal model that the typhimurium people and the paratyphoid people work on.

In the typhoid fever model the lowest reported infectious dose I was able to find for people is ten cfu. The lowest reported dose for SE in people is 28 cfu.

Now, this figure of the 28 cfu causing significant substantial human illness comes from the Schwann's Ice Cream break where they had ice cream that had been nicely frozen so your bacteria is not growing, and they were actually able to calculate what the dose was for people.

Finally, if you look in the literature -- not finally yet -- you will see that *enteritidis* is very good at causing septicemia and deeper tissue problems in people -- osteomyelitis, meningitis, kidney infections, almost any organ you can think of SE can cause these horrible, horrible infections, and in studies where they have compared presenting signs of gastrointestinal illness versus septicemia *enteritidis* is skewed toward causing septicemia just like typhoid.

So when you have -- just to remind you that in the typhoid model gastroenteritis is not the presenting sign, and it's very difficult to find in the environment, much like SE.

People often wind up presenting with typhoid because they have collapsed from anemia, which we can induce anemia in our bird model also.

Birds we cannot get a fever. I have tried getting a fever, I can't get a fever in birds, but of course in people a high fever is quite prominent.

Finally, we have been able to take this cluster analysis of LPS structure and come up with an actually genomic subtyping method that identifies these different clusters and virulence parameters.

Now, we are going to be doing this in coordination with the Ministry for Agriculture, Fisheries, and Food in the U.K. Ernesto Liebman developed this fine map RFLP pattern to analyze different strains of SE on a base pair, looking for single base pair differences, and what we know now is that the things coming out of the mice -- and David Hensler has sent me lots and lots of mouse isolets -- but things coming out of the mice have a very standard sort of pattern here, with the biggest difference being whether or not the strain came from intestines or from spleen.

Here are two spleen isolates here, and the only band difference that we can really detect is right here.

Now, if you look at strains that are orally invasive, which is marked by these two lanes right here, what you see is if it's gotten into the spleen of the mouse then it now has a band produced quite well by these two strains which were orally invasive and egg contaminating.

Finally, my parenteral, my wildtype strain has characteristics that indicate it's a bit unusual, but it shares characteristics with the orally-invasive strain, some characteristics with the mouse strains, and if you line them all up what you find is that this orally-invasive egg contaminating strain has characteristics or bands shared with both mouse isolates and with the parenterally adapted forms. So we're quite excited about finally getting a highly repeatable genomic subtyping method that lets us look at LPS structure, lets us correlate to what we're seeing with our LPS cluster analysis, our virulence analysis in chickens, and also come up with genomic patterns in a simple technique that we hope we're going to be able to disseminate to say clinical laboratories for doing typing, and to get away from just phage typing, which is still a powerful technique.

So anyway, those are some research needs that I see.

MR. BRACKETT: Okay. At this time we will I guess entertain questions for either of the speakers, either Jean or Richard. Do we have any questions for either of the last two speakers?

[No response.]

MR. BRACKETT: Okay. Well, that puts us close to where we had intended to end this morning's session.

We will begin again after our lunch break at one o'clock, and then address some of the research needs from various other aspects aside from what has been identified in the action plan.

So have a good lunch.

[The lunch recess is taken.]

AFTERNOON SESSION

MR. BRACKETT: If we could get our seats again we will get started with the afternoon session.

We designed the different sessions for different purposes. This morning's discussions really revolved around as I said the actual Egg Action Plan, but it's also important to find out opinions and needs outside of that, and so really that's what this afternoon's speakers will address.

Our first speaker of the afternoon will provide an industry perspective of research needs with regard to SE, and we'll have for that Charlie Beard who is with the U.S. Poultry & Egg Association, and has been working with SE for many years.

STATEMENT OF CHARLIE BEARD, U.S. POULTRY & EGG ASSOCIATION

DR. BEARD: Thank you, Bob.

I have been around long enough to know that at one o'clock after lunch you do not turn the lights out, so there will be no power point, no slides, and no overheads. So I've got you guys, you've got to keep awake here.

Talking about working with SE a long time, Bob, I remember that infamous day when I got a phone call from CDC, and they said "We have some people over here that would like to talk with you," and so they came over to Athens, and they sat down in our little conference room, and they presented the story of *salmonella enteritidis* related to eggs that was unfolding at that time, and after they left I called Al Pope, and I called Harold Ford, and I said "You guys are going to be in for a rough ride." Do you remember that, Al?

MR. POPE: Oh, I remember that. You said "Hold onto your seat."

DR. BEARD: I said "Hold onto your seat, I've got news," because it was really a shock to a lot of people.

MR. POPE: It's kept me in a job for ten years now.

DR. BEARD: Yeah. A lot of us are still working off of that one.

But before I comment on what I believe are the important research priorities related to *salmonella enteritidis* in eggs, I want to put a little history on the table that may help us appreciate where we were, what has been accomplished, and where we need to go.

The attitude of the egg industry has undergone a series of adjustments since the SE problem first came to light in 1987. At first there was disbelief, absolute total disbelief. The deposition of SE in eggs produced by normal-appearing hens was counter to all we knew about the association of egg consumption with *salmonellosis* in humans.

The *salmonellae* had not been significantly related to the eating of eggs, or egg-containing dishes since the implementation of the Egg Products Act of 1971 which prohibited the sale of dirty or cracked eggs, and mandated the pasteurization of liquid product.

It would be logical, then, that the first SE research efforts were to determine if hens colonized with SE could lay eggs internally contaminated with SE. If so, when would they be laid relative to the hen inoculation?

How many bacteria would be in a contaminated egg, what percentage of the eggs would be contaminated?

Where would the bacteria be located?

Would they replicate in the presence of naturally-occurring bacterial inhibitors in the egg?

How would replication be influenced by storage temperature?

Were all the SE the same in their ability to result in egg contamination? Was there a difference among the phage types, or within phage types?

Could we predict the behavior of an SE isolate in a flock, especially as it related to egg contamination?

Could the presence of circulating antibodies be used to identify infected flocks?

What about the presence of yolk antibodies?

Will oral emulsion vaccines provide an acceptable level of protection, especially related to egg contamination?

What effects would the stress of molting have on the problem?

There were many unanswered questions, and it was an exciting time. It is not often that researchers have such an open playing field of problems to work on, uncluttered by the fumbles and dogma left by early workers. Unfortunately, neither was there much in the way of research findings on which to build this new effort.

There was significant research successes, and there were failures. There was a lot of communication and shared good will as researchers tried to get ahead of the problem that seemed to get bigger and bigger with each passing week.

We had informal and very candid meetings of involved individuals at the Southeast Poultry Research Laboratory in Athens, and in Pennsylvania at the New Bolton Center.

A unique aspect of the problem was that as a research effort was being organized and implemented a human illness trace-back program with diversion of eggs to pasteurization was in process. There was also a prevention control effort in the Pennsylvania egg industry with USDA APHIS leadership. The pressure for answers from the researchers' efforts was intense and continuous. The challenge was always not to let the regulatory efforts get ahead of the science needed to support it.

The Pennsylvania Pilot Project yielded important information on the association of high rodent populations with infected flocks, and on the cleaning and disinfection of contaminated premises. Their drag-swab sampling of layer houses was the foundation of determining the SE status of flocks, and it is still relied upon today for that purpose.

Many of the questions mentioned above have been answered, albeit some only partially. We have made progress, but there are many questions remaining. I am going to present some in no special priority order that I hope will help us achieve a further understanding of SE in layers which will one day lead to ending the association of egg consumption and SE illnesses.

While there can be some progress made in the laboratory, most of these questions will best be answered in field flocks, flocks that have been found to be SE positive and their eggs diverted to pasteurization could be used for some of this work with no public health risk, and without any increased potential liability on the part of the researchers.

Such an approach would require convincing industry of the need for, and the potential benefits of such a research effort.

Here is the list that is obviously not allinclusive, but done with an attempt to emphasize the problem-solving side of the research issue. And before I give my short list I would like to say that I haven't disagreed with any of the priorities I've heard today. There will be a lot of overlapping with what I have to say. There have been many lists of research priorities through the years, and being a former researcher it's sort of like mom and apple pie, any question needs answered.

Number one, what effect does induced molting have on the incidence of SE in flocks, including the rate and duration of SE deposition in eggs?

If there was an association in realistic conditions with natural exposures in field flocks between molting and deposition in eggs, for what period of time does this increased deposition occur? If it's three weeks after molting those eggs could be diverted to pasteurization. If it's five weeks, after molting, they could be diverted to pasteurization. There could be some control over what happens to those eggs if it is determined there is an increased risk of egg contamination after molting with field challenge. There are a lot of flocks being molted out there, and we should be able to get those data from actual field flocks.

This is of great importance to the industry. If the industry has to give up molting, we're going to have to have about 30 percent more breeder flocks, we're going to have to have 30 percent more hatcheries, 30 percent more killing of male chicks in the hatchery. There are going to be a lot of negative spinoffs; there is going to be a significant economic impact to the industry. So molting is a very, very important question for the industry.

Number two, what is the effectiveness of both live *salmonella typhimurium* and killed SE oil emulsion vaccines on the susceptibility of flocks, including the rate and duration of SE deposition in eggs?

You hear from the vaccine companies that they're wonderful, they really work. You hear from a lot of the users that they rely on them quite heavily. It would be good if some independent researchers not associated with the vaccine companies could evaluate these products with field challenge in real live situations, perhaps where one house the pullets would be vaccinated, the other house would not; good, controlled studies. And I know they're going to be difficult to do, but the industry really needs this information.

There are situations in the industry where vaccine is probably their only hope, their only hope. You can't use some of the proposed procedures for getting rid of SE in many of these situations without totally putting the company out of business. Vaccines really need to be evaluated, and the independently-acquired information needs to be available to the industry.

Number three, do all SE isolates behave alike? Are there methods to distinguish hot isolates from others, identifying SE flocks that should have their eggs diverted, and others not? Is there a difference?

If you find an environmentally positive house and the organism is examined, is there a marker that could tell you "Well, this house is SE positive environmentally, but there's a very low probability of any deposition in eggs." On the other hand you would say with others "There's a very high probability of deposition in eggs." Does such a marker exist? It be wonderful if we had that information.

What is the storage temperature influence on the number of SE bacterial cells over time in naturally-contaminated eggs?

I look with great concern on data acquired on storage temperature influences on artificially-inoculated eggs.

We've heard a lot of discussion this morning about the organisms being in the yolk. I don't think the organism is in the yolk. The organism may be on the exterior of the yolk, on the yolk membrane. It's been my experience it's not in the yolk. But what is the effect of storage temperature?

The new regulation coming down the pike places great emphasis on storage. If the thinking, current thinking of FDA FSIS comes to pass in the regulation, they are going to require storage at 45 degrees of eggs within 36 hours of lay. That will mean that all contract grower eggs will have to be picked up on a daily basis. That will mean that in-line operations will probably have to process eggs every day. There's going to be a lot of economic impact, and a lot of human impact from this regulation based on the temperature of storage of eggs.

You read Humphrey's data, Humphrey's information was generated with phage type 4. They were naturally-infected flocks. We need that kind of information in the United States with our SE. They are apparently quite different from those that were occurring in England, and I don't think we can really extrapolate it too well, but we need this information because it's going to be a significant impact on the industry, this whole matter of storage temperature, and so we need to have a good scientific basis for implementing it. If there's no real scientific basis for implementing it, we shouldn't implement it. It should be acquired with naturally-infected eggs, and again that's going to be a formidable challenge. But artificial inoculation of eggs may not parallel what's going to happen in nature.

Number five, can there be less labor-intensive and more rapid methods to determine the SE status of large numbers of eggs? Doug Waltman dealt with that very well this morning already. Many of these questions have already been asked this morning, and it's interesting how over the years we are still asking the same questions and we haven't really answered them.

It may just be that we don't have enough people working on these problems. You take something with the regulatory impact that SE is about to have on the industry and look at the number of researchers studying the problem, and there's great imbalance, great imbalance.

If this problem is that severe a public health problem you would think we would have more research effort in that direction.

But if we're going to implement the amount of testing of environments, of eggs that the new regulation supposedly is going to contain, we're going to need some shortcuts, we're going to need some improved methodologies, or these laboratories are just going to sink under the load of the increased testing.

Number six, what are the dynamics of SE infections in layer houses? You heard that addressed earlier as well. How is it spread through the house, and how rapidly? How readily does it move from house to house in a complex? How does the environmental sampling result relate to the actual extent of infected hens and the rate of contaminated eggs? Is there a temporal relationship of egg contamination to the first evidence of infection?

I'm always astounded that a huge complex can be sampled with numerous drag swabs, if there is one isolate, one colony of SE out of that house that house is a positive house. We really need to know what that means, we really need to know how it will change. Will it be there a week later, or two weeks later, or will it become more prevalent in that house? We need to know the dynamics of SE infections in naturally-infected houses.

Number seven, we need to develop innovative intervention strategies that will solve this problem short of forcing egg operations out of business. That's a pretty heavy statement. We need to correct the problem without forcing egg operations out of business.

Special emphasis should be directed toward these larger in-line operations with multiple houses of different ages connected to a processing facility by a common head house. These facilities do not lend themselves to effective cleaning and disinfection, and there is very close proximity among the houses with shared workers.

Number eight, all egg-related SE illnesses may not be due to internally-contaminated eggs. When I say egg-related illnesses, I mean those officially characterized as such.

There should be a survey of restaurant and institutional mice to determine if they could be the source of food preparation SE contamination and resulting illnesses. Since the success of the anticipated new SE regulation is going to be based on the number of human SE illnesses, this could be a very important survey for the objective assessment of the effectiveness of that regulation which will be primarily targeting the egg industry.

The SE in eggs experience that some of us have observed from its beginning has resulted in many frustrations and inequities for the industry. First as a source of frustration the regulatory program was implemented before we had the necessary science to support it.

The industry was looked at as if they had somehow done something terrible that had to be corrected, and no one was capable of telling them where they even got SE, how they could keep from getting SE in the future, or how they could assuredly get rid of it and still stay in business. There were many more questions than answers.

That relationship between questions and answers has improved, but not by much. That has led to much frustration. The government is turning up the regulatory heat, but can't provide the needed answers on how to avoid or correct the problem.

The inequity comes from a trace-back-based diversion program. We talked about frustration, now we're talking about inequity. When eggs are SE positive and not abused in preparation there is usually no illness, and therefore no trace-back. I'll repeat that. When eggs are SE positive and not abused in preparation, there is usually no illness and therefore no trace-back.

When groups of eggs containing some SE-positive eggs are abused and not cooked properly, there can be illness and resulting trace-backs with severe economic penalty. Such a system has led to obvious inequities, the extent of which is related to the pasteurization capability or geographic location of the affected company.

There are some places in the United States where there is no pasteurization capability -- Hawaii for example. There are other locations in this country with no pasteurization facilities in the close proximity. There are other companies that have their own pasteurization capability, and it poses no real inconvenience; they just divert eggs from the environmentally-positive house to the pasteurization plant, and they just move their eggs around internally within the company, and they don't suffer any great loss.

There are other companies with no pasteurization capability that have to sell their eggs that are diverted on the open market, and needless to say the pasteurizer knows that, this is a free market system, and so the person having to sell those eggs to a reasonably-located pasteurizer, if the plant has the capacity to take them, is pretty much at the mercy of the pasteurizing operator. So there is a lot of inequity associated with the diversion requirement for eggs.

We haven't been able to tell an egg complex owner where his SE came from, how to prevent it, or even how to transition to negative status without going out of business. We're just demanding that he fix the problem as if he were General Motors, or Boeing, or Bidgestone/Firestone.

We owe these people good scientific data that they can use, and it needs to be presented in an understandable form, and that is the challenge for the researcher. We owe these people some answers on how they can prevent and get out of the problem they're in.

I always try to put myself in the shoes of an impacted producer, how can I get rid of SE? How can I be certain I don't get it again? Hopefully everyone that's involved in the SE issue from the researcher on the bench to the regulator in Washington has been on an in-line egg farm with ten 100,000-bird layer houses, each of different ages connected by a head house to a processing facility. You cannot see such an operation without being overwhelmed with the obvious difficulty of cycling the facility from SE positive to SE negative status while staying in business and not losing it all.

All SE researchers and others of us involved should put ourselves in the shoes of egg producers in the morning as we plan our day. The next day we should think of the aged grandparent, or the small child that acquires SE from contaminated eggs with very serious health consequences. If we all did that, we would all work harder, more creatively, and hopefully with more beneficial outcome to all, including those we serve.

All of this has come very close to me in my new position with U.S. Poultry & Egg Association. I had an owner of a billion-bird [sic] layer operation, had ten -- in fact, I think it was 1.2 million -- involved in a trace-back because he had sold eggs to a company that was involved in a trace-back, and they came back to him as the producer.

He called me for my counsel, and he said "Dr. Beard," he says "I'm really in a quandary, because if my flock comes back positive I'm doing away with it, I'm out of business, I'm gone. I am not going to make anybody sick."

It turns out when his results finally did come back his flock was negative, but the stresses of the trace-back had been so great he sold his company; he's out of the egg business, he's in the car business, got a car dealership.

And I couldn't believe it. His wife told me that she had never seen this individual so stressed in his life, and he was about 55 years old. So it's very difficult on these companies, and as we plan our research, as we deal with these issues we need to try to work out a plan of action to provide answers that are very problem-oriented that these people can use. They have no other source than to go to the researchers.

The regulators are telling them to fix it, but the regulators aren't telling them how to fix it. It's up to the researchers to tell them how to fix it.

So that's my message to you. I don't disagree with a thing I've heard today, I'm very impressed with the research progress that's been reported here, with the scientists that have reported it. We just need more of you as I can see it, because the problem is mammoth, and as the regulatory impact takes its toll on the industry there's going to be a crying for assistance, for information on how to correct the problem.

Thank you.

[Applause.]

MR. BRACKETT: Thank you, Charlie. We appreciate that.

Our next topic is one that quite often is -- I wouldn't say necessarily ignored, because we often have consumer representatives talking about the needs, but this is a little bit different perspective that we have arrived here, which is the research end of consumer behavior, and I have asked probably one of the country's most notable experts in that area, Dr. Christine Bruhn from the University of California at Davis to come and address this topic.

STATEMENT OF CHRISTINE M. BRUHN, UNIVERSITY OF CALIFORNIA AT DAVIS

DR. BRUHN: Thank you. I am pleased for the opportunity to share with you consumer attitudes and practices related to the handling of eggs, and looking specifically at what might happen should the public receive an egg that would be contaminated with SE.

So I brought a few copies of my presentation and have given them to our organizer in the front. To begin with I would like to point out that *salmonella* is a word that consumers have heard about, and research done actually in '96 pointed out that a high percentage, 80 percent of the people said they were familiar with that term *salmonella*, and they could correctly identify, half of the people could correctly identify a food which would be a source of *salmonella*.

Furthermore, people when specifically asked were able to indicate that *salmonella* was in their mind associated with poultry and eggs, and in California we found that 84 percent of the people said that they knew that sometimes eggs could be contaminated with *salmonella* and that this would cause an illness.

Nevertheless, people believe that food-borne illness is generally caused by mishandling, by inappropriate sanitation, by food being spoiled, by not cooking food well enough, and they do not perceive that something that is a healthy food, and eggs are viewed as a healthy food, would carry something that could cause illness such as *salmonellosis*.

When people were asked specifically how confident are you in the safety of different foods we see that the confidence in the safety of eggs is relatively high. Notice by making this question we are sensitizing people to tell us that they don't think that they're very confident. The act of asking increases sensitivity, and it increases these numbers, but fruits and vegetables and dairy generate the greatest completely confident, one-third of our population, with eggs following very closely at 28 percent. So eggs are viewed with a great deal of confidence by the public.

And we asked people if you stopped eating, or stopped buying a product in recent years, what was your reason? And again we're sensitizing people, we allow them to check multiple reasons, and we had the primary reason for eggs being cholesterol at 80 percent, and fat content at 30 percent, with only 15 percent saying "I'm worried about bacteria." And once again they could check every box if they wanted to. So eggs are not seen as a heavy source of bacterial contamination.

Consumers follow several handling practices, some of which are recommended and desirable, and others can lead to an increased probability of illness should the egg be contaminated with SE.

One of the things people are supposed to do of course is refrigerate the eggs, and multiple studies indicate that most people do indeed refrigerate their eggs, but some leave the eggs sitting at room temperature for thirty minutes or longer. Does that increase risk? You need to tell me that. But they do follow this practice.

They are also aware that they should not use cracked eggs with 79, almost 80 percent of them saying they do not use cracked eggs. But focus group work that I have been involved in indicates some consumers wonder why that's risky, because the egg industry sometimes is selling them eggs that are cracked, and they wouldn't be selling them something that wasn't safe. So it's hard for them to visualize that the eggs would be more dangerous if they're cracked. They don't use them, but some people don't understand why the concern.

We indicated that they refrigerated eggs, but a study done by Audits International indicates that some people's refrigerators are not as cold as they should be. This was not a random sample, this is a sample of people who primarily have a higher than normal degree of formal education, but we have in total over 30 percent of the people whose refrigerator is over 42 degrees, and 9 percent, or almost 10 whose refrigerator is over 45. Is that a cold enough temperature to raise concerns about SE? You or the microbiologists to respond to that, but consumers don't always keep their refrigerators as cold as some might recommend.

When they were asked specifically why isn't your refrigerator cold? most people, 70 percent said "I was not aware what the standard was." They didn't know where they should be putting their refrigerator. Some said "I didn't think it was very important." That's the motivation aspect of behavior change.

Now, we know that people should be washing their hands, and also washing the counter and the preparation area. This study which was done in California looked at how frequently people did wash, and we found much greater frequency in washing the preparation area before and after handling eggs with about 80 percent indicating that, but only about half the people washed their hands before or after handling the eggs, and people were asked "Did your hands get -- did you touch the egg when you were washing it?," I mean the egg interior, did your hands get wet, and we have again about 50 percent who said that they washed their hands afterwards.

So once more the question might be is it knowledge or is it motivation, and is there a variation between different demographic behavioral things like maybe age or education or gender or something like that between those who do and those who do not wash, and this study was about someone else, and not specifically about eggs, but I think the findings would be likely to be reflective as far as egg-handling. They found an increase in knowledge that people should wash their hands as education increased, with those who had not graduated from high school least aware that washing hands was important, but then when they looked at had self-reported actual washing of hands it didn't vary by education or by other factors such as income. Some people didn't do it even though they knew they were supposed to.

Again, this is all self-reported. We have 67, almost 70 percent self-reporting they washed their hands. If you have a video camera in the kitchen and watch to see if they actually do, you find that people don't wash their hands as frequently as they say they are.

So we have a gap, a profound gap between knowing what you should do and actually following the practice, and that's a very important area I think for research and for the focus of how to increase safe handling.

We asked, or someone asked "Why don't you wash your hands?" and about 60 percent said "I wasn't aware that I should." Now, this -- we don't have the numbers for this, but in focus groups with people in California that particularly became important, "Why should I wash my hands? Eggs are clean, aren't they? So what if I got some of the white on my hands if it's a clean product," not realizing that they were putting a nutritionally-rich product on their fingers which then may be important for other activities.

But people were not aware that it was important to wash their hands before and after handling eggs, and there were also some who did know that they should, but who thought it was not important, so you have again both the motivation and the education as factors.

And then for washing the counters and washing the bowls, once more 65 percent said "I was not aware that contamination could have occurred." This was a general finding, but we did find it specifically for eggs in some of our California work where people would use a mixing bowl to mix something that contained eggs, and then would use that bowl again without washing it. They didn't feel this was a problem because in their views eggs were not carrying anything that they should be worried about, so why wash with soap in between, it really wasn't necessary. If I do anything, maybe I'll rinse it out with water.

We also find people are sometimes consuming raw eggs, and we've got a couple of studies. Here 72 percent in a California-specific study said they never consumed raw eggs, but that leaves you 30 percent who do.

And another study in California, 15 percent of the population had eaten raw eggs within the last thirty days, and this behavior was twice as commonly reported among the Hispanic population as among the nonHispanic population. This lists some of the foods that people were commonly consuming, but as you might expect the raw egg product varied also ethnically, and it's very common among the Hispanic population to put raw eggs into a blended fruit drink, fruit juices, or it could be fruit and milk, but the raw egg adds flavor and in their mind increases the nutritional value of these products.

As far as egg cooking is concerned, we again find a fair percentage of the population if you're just looking at sunny side up and over easy we've got over 50 percent of the population who are not thoroughly cooking their eggs. This group was not asked why, but I bet I know why. I bet it's because they like the taste of them when they're not thoroughly cooked. Right? They enjoy the flavor of the runny yolks which are present here, so that is a driving factor and they don't see this product as a risky product, or they see the risk so low that they are not changing their behavior.

As far as cooking of casseroles or mixed dishes that might contain eggs, most people do not use a thermometer, and this is an Audit International nationwide study found that 20 percent of the people did not follow the recommendations to thoroughly cook their foods to the recommended temperature.

The work we've done in California, I asked specifically if people used thermometers. Very, very few, less than 1 percent used a thermometer in something like a casserole; they judged doneness by was it steaming, was there bubbles around the edge, did it kind of look right by texture or by color, and if it was a deep product and ingredients were cold it is very possible that the edges could look just right and the inside not have reached adequate temperature.

So this is an opportunity where contamination can occur, and it can be exacerbated because some people when they have leftovers they don't thoroughly cook them before they are serving them, and sometimes they judge whether they're safe or not by tasting them, and again in California 26 percent saying they always, and another 21 percent saying sometimes taste leftovers to see if they're safe, and through focus groups I was just amazed to find several people volunteering to me that they don't taste it, they give it to their kids to taste because their kids are very fussy, and if it tastes wrong the kids will pick it up for sure, so they are now exposing what might be one of their high-risk audience to a product that might not be thoroughly cooked.

So this is a myth on taste and safety, and also lack of knowledge of who is at high risk.

Handling labels, will handling labels make a difference. Again, a California study we had 41 percent saying they always, and another 24 percent saying they sometimes read the labels on products. We have a large majority, 86 percent saying "Oh, yeah, that's a good idea," but only less than 20 percent, or about 20 percent saying that they would change their behavior as a result of handling labels.

Well, this is all theoretical, and we wonder if they really would change behavior. A study relating specifically to this area was commissioned by the California Egg Commission, and I want to share with you then as my last set before I get to general conclusions some of the key findings from this study.

It was based upon focus groups, focus groups first with consumers to develop a way of communicating with the public so that they could grasp the message whether they were literate in English or not. Our state now has a majority of population is Hispanic, not all read English; we have massive other cultural groups as well, and some of them do not read English, and we have kids cooking, so we need to have something that people can understand easily.

Consumers told us the print needed to be large enough for easy reading. Several complained that the meat and poultry guidelines on all the packages was too small to read. They also said that the messages were the same all the time, so they looked it when they first came out, but they don't look at it any more because it's always the same. So they advised us to vary the message. They suggested using contrasting colors and bright colors so it really pops out, and some said we should be innovative and we should use humor.

I wasn't good enough to think up a good humor myself, but one of our focus group participants sat in the back of the room and was kind of, you know, thinking and writing little notes, and he said "You need to follow the same guidelines as the Burma Shave," remember the Burma Shave? Some of us are old enough to remember the Burma

Shaves as you would drive along, and it used to be really a delight as a kid and on a long trip you would always look for those, and he said a different little slip of paper could go inside of every egg carton and, you know, develop lots of things, and over time people would hit all the messages, and because they're funny people would remember.

In any case, we worked with the public to develop some icons, and I'll show those to you in a few minutes. We did use words, but we kept the words to a minimum. Because consumers said our messages should vary we prepared four different labels.

Consumers told us that two things were so important they should be on every label, and that is keeping the eggs refrigerated and washing your hands. But then the other messages we varied, so as I mentioned we have four different labels. I'm going to show you what those labels are.

Keep refrigerated again, but this is the eggs going into the refrigerator, not just the picture of the refrigerator.

Washing your hands before and after handling eggs, but the innovation here was the addition of soap. We found many people did not know they should use soap; they thought getting their hands wet was adequate.

This was the make it and then break it, you know, the don't let it sit around for thirty minutes or longer before you prepare it, but make it and break it right away.

And then California has got a lot of Hispanics. We found in our focus groups that many people were eating the raw eggs in a blended drink, so this was don't eat raw eggs, you know, as you see breaking it over the counter.

Here's the next labels, and again the first two messages are the same. Now we want to have people to wash dishes, and then to cook it to 160. They said most people don't use thermometers. This label was not remembered very well because people don't relate to it.

Here's our next set with the innovation here of the two new ones, it's cook eggs thoroughly, we're trying to get a little kid and an older guy -- I don't think that really came across, but it was at least indicating for all of our family.

And then don't use cracked eggs. This was the most universal symbol that everybody came up with. They wanted to have this red, they wanted that ring to be red over the cracked egg.

And then the last set, we had nine messages where we had to repeat one thing, and we repeated cook to 160 because it gets the concept of thoroughness, but we tell people how long then can keep the eggs. Most people haven't ever told them that. In fact, I had several people coming up to me saying "I never knew how long was wise to keep the eggs, I never noticed that there was date at the end of the package." So the length of the storage time is a very important innovation for our consumers.

Now just for the last three slides I'm going to show you how people responded to these messages. The labels that were most frequently remembered were the keep refrigerated and the wash the hands, but the don't eat the raw eggs and the use of cracked eggs, and use within three weeks of the sell-by date were also remembered very thoroughly.

By remembered I should indicate we had people come together, asked them questions about how they handled eggs, then gave them an egg carton with these handling guidelines right on the top, and then we asked them to come back, made appointments two to three weeks later, they came back and we asked them "We gave you some eggs last week. Did you notice anything different about them?" or the last time, and "What did you notice?" And we had people recalling that there were handling guidelines. I mean they would have to be blind not to, but they did remember it at least.

And then we asked them to tell us what some of those were, so this is not us prompting, it's drawing on their memory. So this is what they remembered they saw.

Then we asked them some of the same cooking, how you handle and how you cook questions that we had asked before, and we had an increase in people who reported to us that they cooked their eggs firm, up to 70 percent from around 50.

We had a decrease in consumption of eggs raw, but this was only about a two- to three-week period, and it could be they didn't have the occasion to have some of their favorite raw egg products.

We had a small increase in washing of utensils, we had no change in frequency of hand washing.

The consumers made some suggestions to us. This was a verbal personal interaction so we were able to write these down, and also during the focus groups they said guidelines alone are not effective, you need to have a comprehensive educational program, and you need -- some of the people did not believe the guidelines; they did not believe they should wash their hands after the eggs after the eggs, they did not believe they could not eat raw eggs.

Especially among the Hispanic culture raw eggs were viewed by these individuals as very health-promoting products. If someone is sick they put them to bed and give them a drink with raw eggs. If a man or a woman wishes to start a family or have a child, either one will eat a raw egg straight, and I had testimonials about how effective that was. It increases your virulence [sic], it increases your health, it will help you recover if you're ill, so they just could not believe that it wasn't a good idea to eat a raw egg.

So it's important to explain why the guidelines are important, handling especially. They didn't understand the idea about the soap because they thought they were doing fine, their hands already looked clean, they used water, wiped it off, what's the big deal about soap.

And many suggested developing a safe program for children for the schools, because not only will you teach the next generation, but the kids will bring it home, and this came up frequently especially in the Hispanic community, but it was true for all that they thought this was a cool idea.

So you have been speaking about to prevent the SE from getting in the egg, from my perspective I look at how you get the message to the people that this is a concern, and how you get them to act on it.

So if you can't give a *salmonella*-free egg to the public, then it would be wise to tell the public how serious SE is, to tell them who is at greatest risk, to explain to them how a healthy food like eggs can carry a bacteria which is dangerous. We don't want to make them so frightened about this healthy food that they avoid a good-tasting, nutritious, functionally valuable product, and then to target the message to specific cultural groups depending upon the practices they are already following, and I gave you examples of that with what some of the Hispanics are doing.

Then for the content of that message the personal sanitation and kitchen sanitation, to use the soap actually towels also instead of frequently-used dish cloths would be another thing to target.

Refrigeration, consumers recognize, yeah, they should probably refrigerate it, but they refrigerate it more because they are in the habit of refrigeration, and they really wonder why retailers don't always refrigerate.

Now, I know maybe retailers should refrigerate, but if they're having an egg promotion you'll go into a retail store and you will find stacks and stacks of eggs in the milk carton cases stacked outside of the refrigerated egg display area, because they are expecting a big run, and they don't want to repeat all the time, and this is not the corner store, this can be a mainline chain grocery store and they're not refrigerating their eggs. So that's sending a mixed message to the public. If refrigeration is important it should be followed by all parties.

Reasons for thorough cooking. It's important to provide pasteurized eggs again because some people are not going to believe you and they're going to continue to eat the raw product, so either remove the -- be sure you have no SE in there, or you give them a pasteurized egg so they can use their favorite dishes.

And then finally many of the outbreaks are related to what happens in food service, so how can the consumer judge if the restaurant they're going to is handling the eggs as safely as they are. Boy, that's a real challenge, but can there be some guidelines. Consumers always ask me, reporters ask me "How can I tell if I'm going to a good restaurant?" Well, how can they? Are there some things that we could say as health professionals to guide them in their selection of a place so they will not be putting their family at risk when they go out for a sociable and pleasurable activity.

I think that's it. Thank you very much.

[Applause.]

MR. BRACKETT: Thank you, Christine.

Our final in the series of presentations is going to be given by Eric Ebel who was one of the primary authors of the SE risk assessment done by USDA, and this has been mentioned several times, and if you've ever been involved in these risk assessments you know there's as much revealed that you don't know as there is that you do, and so Dr. Ebel will tell us what research gaps were identified in the SE risk assessment.

STATEMENT OF ERIC EBEL, USDA

DR. EBEL: Thank you. I do want to acknowledge another of the authors of this risk assessment, in fact one of the leaders, Roberta Morales, who happens to be in the audience today.

But in mid-1998 the FSIS and FDA released a report describing a risk assessment model for *salmonella enteritidis* in eggs. This model estimates the baseline risk of human illnesses associated with consuming SE-contaminated egg meals. Today I want to discuss some research implications of this SE risk assessment.

Before getting started, I think it's important to review why food safety risk assessments are needed. A fundamental purpose of these risk assessments is to summarize what is already known about a pathogen in foods. No other technique is quite as rigorous as risk assessment in pulling together disparate evidence and putting it all in one place for interpretation.

Typically we want to summarize the science about a problem because we want to control the problem. Risk assessments provide decision-makers with a tool for evaluating the public health benefits of various control options.

Nevertheless, decisions about control are difficult because model inputs can be very uncertain, and that uncertainty is propagated to the models' outputs. Therefore, oftentimes the most valuable contribution a risk assessment can make to problem solving is by identifying data gaps and prioritizing research needs.

For this presentation I want to distinguish between data gaps and research needs. I am defining data gaps as hypothetical factors that might be influential in modeling a pathogen in a food commodity, but do not yet have sufficient scientific support; therefore, they are not included in the model. These data gaps are identified during the process of reviewing the available evidence prior to constructing a risk assessment model.

In contrast to data gaps, research needs are identified by analyzing factors that are explicitly in the risk assessment model. In other words, research needs address model inputs for which some scientific evidence already exists, but more research is needed.

Research needs are generated by considering the intersection of importance and uncertainty. Important inputs are those whose control would substantially reduce the number of human exposures or cases occurring annually.

Uncertain inputs are those that are based on limited data, and therefore not precisely known.

Research priorities can be ranked by the elements of importance and uncertainty. An important input that is also highly uncertain is clearly a research need of high priority. In contrast, unimportant inputs that are highly certain are clearly not worth researching further.

Now, this is a diagram of the five modules that make up the SE risk assessment. The five modules are linked together to show how eggs flow from the farm to the consumer. The production module simulates SE-contaminated eggs produced by infected flocks.

The shell egg processing module simulates the period between egg laying and arrival of eggs at retail or wholesale distributors.

The preparation and consumption module simulates the storage, preparation, cooking, and consumption of egg meals.

Finally, the public health module predicts human illnesses as a function of dose of SE ingested.

Given the emphasis of today's meeting I'm going to focus on the data gaps and research needs generated from the production and shell egg processing modules.

For the sake of completeness, however, I will list some prominent needs from the other segments of the model at the end of the presentation.

Now, here are some average results from the baseline SE risk assessment model. The production module predicts that about one in every 20,000 eggs produced are

SE-contaminated, and that most contaminated eggs contain less than 40 SE organisms.

The shell egg processing module does not predict any increase in the numbers of SE within contaminated eggs. This is because the lag period for these bacteria is not entirely used up during the processing stage. Nevertheless, an average of 25 percent of the lag period is expended during this stage.

The preparation and consumption module predicts that less than 10 percent of contaminated eggs experience any increase in SE numbers before cooking. Furthermore, this module predicts that SE is entirely eliminated from about three-quarters of contaminated servings after cooking.

On average the model predicts there are about 2.7 million contaminated servings per year that result in about 661,000 human illnesses, so about 25 percent of simulated exposures lead to illness in some form.

Inputs to the production module are used to predict the prevalence of all flocks that are infected with SE. SE-infected flocks are further stratified into high, moderate, and low within-flock prevalence classes.

Infected flocks are further dichotomized into molted and unmolted flocks. For each type of infected flock a frequency of SE-contaminated eggs is then applied. Most of the evidence used to estimate these inputs came from national surveys and the Pennsylvania Pilot Project that was conducted between 1992 and 1994.

Here is an importance analysis of the production module inputs. Importance analysis demonstrates how various inputs influence human exposures. In this case high prevalence flocks produce on average about two-thirds of all SE-contaminated eggs per year, despite only representing about 11 percent of the infected flocks.

Moderate prevalence flocks comprise about one-third of the contaminated eggs, but two-thirds of the infected flocks.

In contrast, low prevalence flocks contribute a minuscule proportion of contaminated eggs, but represent over 20 percent of infected flocks.

Uncertainty about inputs is represented by probability distributions in the model. We completed sensitivity analysis to evaluate the effect of input uncertainty on the predicted number of contaminated eggs per year.

Correlation coefficients measure the degree of sensitivity of the model to various inputs. Our analysis shows that uncertainty in egg contamination frequencies for high prevalence and moderate prevalence flocks is strongly correlated with the predicted number of contaminated eggs per year.

The model is also sensitive to uncertainty in the prevalence of infected flocks, as well as the frequency of high prevalence flocks. It is somewhat less sensitive to uncertainty in molting and flock testing inputs.

Now, if we prioritize research based on the intersection of important and uncertain inputs, then within-flock prevalence factors are clearly priority research needs. These variables were developed from limited Pennsylvania Pilot Project data, and may not be representative of all U.S. flocks or regions.

Furthermore, these data were cross-sectional, so more research is needed to evaluate changes in within-flock prevalence and egg contamination frequency across time.

Although egg contamination frequencies were estimated from a large amount of data, they are also important in uncertain inputs to the model.

They are uncertainty results because most of the data came from Pennsylvania flocks. The sensitivity of environmental testing and the duration of time that molted flocks produce contaminated eggs more frequently than unmolted flocks were other model variables that might warrant additional research.

While research needs were generated from analysis of the model, data gaps occurred before the model was even built. Because these data gaps could not be included in the model we can't evaluate their importance.

One data gap was the need to understand the relative importance of various routes of introduction of SE into commercial flocks. Studies are needed to quantify the relative importance of carryover infection between flocks, introduction of infected pullets, rodent reservoirs, as well as other risk factors that might predispose flocks to infection.

Advocacy studies concerning vaccination of flocks, rodent control in and around layer houses, cleaning and disinfection of layer houses, and competitive exclusion were also identified as data gaps.

Other gaps which could be investigated in future research projects include the association between severity of SE infection and specific strains of SE, the geographic diversity of SE egg contamination frequencies, and the efficacy of various molting strategies on SE infection.

Random surveys of eggs for the presence and concentration of SE bacteria are also needed to validate the numbers obtained from this and future models. These surveys should occur on a national basis.

The shell egg processing and distribution module actually models what happens to an SE-contaminated egg from the time it is laid until it is delivered to and end user. Shell eggs are stored, washed, packaged, and transported within this module.

SE-contaminated eggs enter this module with a certain number of SE organisms. The temperatures these eggs are exposed to and the time these eggs experience different temperatures determines whether SE grows in eggs in this module.

Predictive microbiology equations are used to estimate the rate at which yolk membrane integrity is compromised, as well as the rate of growth once growth commences.

Ambient temperatures influence internal egg temperatures, and this effect is modeled through heat transfer equations which account for different packaging materials through the use of pooling concepts.

To illustrate the general importance of the shell egg processing module scenarios were considered where ambient temperature was fixed at 45 degrees Fahrenheit throughout this stage. On average, 8 percent of human cases were avoided by this mitigation strategy. An additional 4 percent of human cases were foregone if eggs were immediately cooled to 45 degrees Fahrenheit after lay.

Sensitivity analysis of this module's inputs shows that uncertainty regarding storage times and temperatures is correlated with the output of this module.

Results of importance and uncertainty analysis for the shell egg processing module demonstrate that more research is needed on storage times and temperatures.

Albumen is generally an excellent inhibitor of SE growth. This inhibition is maintained until the yolk membrane loses its ability to keep apart the SE in the albumen and the yolk contents.

The time to yolk membrane breakdown depends on the storage temperature. Typical values are seventeen days before yolk membrane breakdown when the egg is stored at 68 degrees Fahrenheit, and only four days before yolk membrane breakdown when the egg is stored at 95 degrees Fahrenheit. This essential information comes from a single study. This study needs to be validated.

Growth rate equations are also based on limited data, and need further research.

It would be useful to predict the internal temperature of an egg at a specified time given the initial temperature of the egg, the ambient air temperature, and the packaging characteristics. Only a few cooling curves have been published on the internal temperature of the egg over time, and no modeling or engineering studies are available.

Studies are needed which correlate the internal egg temperature to the type of packaging material used, the position of the egg in a pallet of stacked cartons of eggs, and the ambient storage temperature.

A research need that spans both the production and shell egg processing modules concerns the starting numbers of SE in contaminated eggs. There are only two studies that measure the numbers of SE inside eggs at the time of lay. These studies involve the enumeration of a total of just over sixty contaminated eggs.

Furthermore, these studies do not agree very well. The limited data and conflicting results indicate that more research is needed to quantify the numbers of SE bacteria inside contaminated eggs. It is preferable that these studies be conducted with naturally-infected eggs.

Several research needs and data gaps were identified for the egg products processing module. I'll just list them here.

Examples included the sources and numbers of SE in unpasteurized liquid eggs, the variability and efficacy of pasteurization, and the occurrence of different pH levels in commercially-processed albumen.

There was a great deal of uncertainty associated with inputs used to construct the preparation and consumption module. This module was the most complex of all the risk assessment stages. Research needs and data gaps identified from this module included the distributions for storage times and temperatures in different settings, data on egg pooling practices and the degree of cooking and efficacy of cooking applied to egg meals.

The public health effects module also identified research needs and data gaps. These included studies that estimate the susceptible proportion of the human population, data for use in modeling dose response relationships, and more epidemiologic research concerning SE illness in humans.

In conclusion, research and risk assessment should be recognized as mutually dependent on one another. Because risk assessments are decision tools that link policymaking to science they depend on scientific research.

Furthermore, researchers are increasingly required to demonstrate the utility of their proposals to policymaking. Consequently, researchers benefit from the findings of risk assessments, especially the research needs generated by risk assessments.

The processes of research and risk assessment are iterative and feed back on one another. Filling the gaps identified by the SE risk assessment should improve future risk assessments, as well as endow future research with greater relevance to solving the problem of SE in eggs.

This completes my presentation. I will be glad to answer any questions.

[Applause.]

MR. BRACKETT: Thank you, Dr. Ebel.

It is now time for a break again. We are a little bit behind schedule, but not too bad. But we do have some big, fat cookies in the back as well as some cold soft drinks, so let's plan to be back here in fifteen minutes and we'll have the panel discussion at that time.

[A brief recess.]

MR. BRACKETT: It's two-thirty, so if we could have the panelists come up and take their seats. I would like to keep as much as possible on schedule because there are people who do have to catch airplanes this afternoon yet.

This portion of the meeting is actually somewhat of a synthesis of what has come before, and the purpose of the panel discussion actually is twofold, one of which is to try to get some consensus on where we are and where we need to go, but secondly also to hopefully stimulate some dialogue on really what the needs are.

One of the things that we have done is try to come up with some different questions on how a meeting like this would best contribute to the process of doing, as Dr. Beard said, of actually doing something about the problem, and getting a group like this together and then talking about this is I think one of the steps forward.

So really we have three questions that we at FDA would like to know, and we'll start off first by asking the panel members, and once they are finished if others in the audience would like to make a statement to answer one of these questions briefly that will be considered as well. Again, if you do talk, please state your name and your affiliation.

One of the first questions that we had, and I think it has become actually more difficult with all of the good information that we've gotten today, that we have received, is what -- and this is a question to the panelists -- what would you consider to be the priority needs? and by this case I would say that if we can come up with even a top three needs that would be helpful in setting some priorities for research funding as well in the future.

I guess I'll start off in the order that we went through. Peter.

DR. HOLT: You're kidding me. Priority needs. Well, I would have to say molting would probably be --

[Laughter.]

DR. HOLT: Gee, it's difficult to really set one priority over another. I think that, to tell you the truth I think Eric may be the one to start it off, because he's the one that did all the risk assessment, and I think he knows where the gaps really are better than I would.

MR. BRACKETT: I'll take your suggestion. Eric, go ahead. What did the assessment say?

DR. EBEL: This is Eric Ebel.

The risk assessment doesn't have a definitive answer. Primarily because of the complexity of the model we had to evaluate importance and uncertainty within each of the modules, so in the way the presentation I just gave was laid out we could talk about individual modules, and in an attempt to rank them go through those inputs and evaluate them with regard to importance and uncertainty.

But based on first of all the sensitivity of the model to what we think is going on in terms of total numbers of eggs that are being produced annually that are contaminated, it does point to the need for evaluation of flock status, and if we go back to the production module the thing that keeps coming up as very important is trying to distinguish among those infected flocks, those that may be responsible for a disproportionate part of the problem. In other words, not all infected flocks are the same at least in a cross-sectional sense.

What we don't know is if all flocks are the same in the sense that they temporally change through their lives, but what we see in the data so far, and the only evidence that I could bring to bear on that was the Hensler-Sisco paper that evaluated Pennsylvania Pilot Project flocks and found that of those flocks with heavy doses or high numbers of positive environmental samples out of the numbers of samples that were collected in the flocks, those with greater than 50 percent of those samples positive environmentally also were the ones that tended to have the higher egg contamination frequencies, so that correlation suggests that at least in a cross-sectional sense there are differences in flocks, and I would say that the research priority should be in verifying those findings elsewhere, and evaluating then factors that might explain why some flocks produce eggs at higher frequencies than others, or why flocks produce higher frequencies of contaminated eggs at certain times.

And so that's where I would put my priority.

MR. BRACKETT: Okay. So would I be correct in summarizing this by saying identification and verification of flock status? Does that capture it?

DR. EBEL: I would say it's identifying and characterizing the distribution of severity of infection where we measure severity on the basis of egg contamination frequencies.

MR. BRACKETT: Okay. We'll work our way back this way. Christine, did you have any input from a different perspective?

DR. BRUHN: I would think that you shouldn't just work in one area, but his model had four areas, didn't you? You had four in that first graphic, you had four items. So I think you have to do something within each of the four areas, and you can't just put it in one spot, and I believe you need to identify the most important priority in each of those four so that you can move together in a more comprehensive fashion.

MR. BRACKETT: Okay.

DR. BRUHN: And within the consumer area as I mentioned I think motivation to follow what you know is right is probably the most challenging of the research priorities, how do you motivate people.

MR. BRACKETT: Okay. Charlie, you had a whole long list. What do you consider to be the primary need or weakness?

DR. BEARD: Relative to the proposed regulation, upcoming regulation, nothing is really more important than establishing a scientific base on the time, temperature of storage factor. That's going to be a very costly portion of the regulation, and there needs to be a defensible scientific base for requiring the implementation of something like that.

The other research need that I see representing the industry is a need for an intervention strategy so that an operation can, number one, prevent SE, and, number two, convert from positive to negative status, and I emphasize the importance of a third-party vaccine evaluation of all available vaccines on that.

Some countries like Germany are already requiring immunization of layer flocks, and those people aren't stupid, so there must be some rationale for that, and we've got to look down the road and try to provide for people that for some reason get infected an avenue of getting out of that ditch, and we haven't done that.

MR. BRACKETT: Okay. Thank you, Charlie.

Jean.

DR. GUARD-PETTER: Hi. I'm Jean Petter from USDA ARS in Athens, Georgia.

I would concur with Charlie's comments on the need for objective vaccination trials. I have worked with some companies on their vaccines; I have found lot-to-lot variation in killed vaccines which means they may differ in their efficacy depending on what lot goes out.

Modified lives need a very hard look. We have submitted a paper on the failure of the modified live to prevent egg contamination specifically, even though it met other label claims, so this is a real concern of mine is that the SE problem is very different from the typhimurium problem, and there's not actually a modified live licensed for use for aiding the reduction of SE. There's a typhimurium vaccine, the Megan product licensed for use in the young growing birds, so the *enteritidis* people, people who have laying flocks are using it, but they justify using it in that they will only give it up until the bird becomes mature, and they claim they're meeting the label restrictions.

So this is -- we do not have vaccines modified lives that have really been tested for their ability to stop or reduce egg contamination.

There are some -- I personally am quite excited about the idea of at least taking a hard look at egg shell quality to see if it can be used at all to predict clusters of contaminated eggs, or to perhaps use as a warning sign that maybe some eggs are sneaking through the grading process.

The ability to apply this would be fairly cheap, it would be high throughput. It's conceivable every egg could be scanned because it's all digital output data that basically would need a computer set up and somebody listening for the bell that goes "Beep, there goes a bad cluster of eggs, maybe we had better take a look at them."

I'm not saying it will necessarily identify the contaminated eggs right then, and that's where I see a research need is just to explore that issue about what sort of correlation might exist between shell quality as an indicator of perhaps recent active infection, SE infection in hens.

I also personally think the SE program has suffered by not having a geneticist assigned to it. I have fought for this, I have written grants and hired geneticists, and I've got to tell you almost all of my work the past three years has required a geneticist's input, and I'm surprised really today that we still don't have an SE geneticist. I'm not talking about a molecular biologist, I'm talking about someone who knows a gram negative bacterial chromosome backwards and forwards and knows how to really manipulate it, because that's where your markers are going to come from strain heterogeneity, and for virulence factors, so I think those three -- and it's actually areas that I'm already working on now, but I keep bumping up against the limits of my own research program and, you know, can't expand past that. So let me pass this.

MR. BRACKETT: Charlie, another comment?

DR. BEARD: Bob, when you ask any good researcher for the top priority research item, if they don't list their own research I'm disappointed in them. Jean is no exception.

But, Jean, I have to take exception to your proposal that egg shell quality should be a high research priority. There are so many nutritional and disease factors, and age factors that can influence egg shell quality that may be a long shot, but it is a very long shot, and it would be very difficult to determine whether it's bronchitis virus, or influenza virus, or nutritional problems, or age, or whatever that influences that egg shell quality, and I wouldn't spend a nickel on that related to SE.

DR. GUARD-PETTER: Well, we disagree, because for one thing it would be fairly inexpensive research to do because the equipment has already been developed and patented, and USDA wouldn't necessarily have to do it. A directed research project to the Griffin, Georgia CFSQE facility might help answer the questions.

And also just -- you could actually run some very low-key experiments to begin asking the question if you can use egg shell quality to see an increase in incidence of contaminated eggs above what the risk assessment model suggests is there.

It's just an alternative approach. People want creative approaches, this is one. We're seeing a role for a specific molecule of virulence in causing the problem, and I personally would spend a nickel on it.

MR. BRACKETT: Richard.

DR. GAST: Actually I thought between Jean and Charlie I was going to get a little more time to work on my cookie.

About two years ago, some of you actually here were involved with it, we put on with the AVA meeting in

Baltimore a symposium on controlling *salmonella* in poultry, and during the planning phases to decide what would be on the program there were an awful lot of potential directions we could have gone, and at the time Charlie had probably been the strongest advocate of the idea that what would be most valuable to the poultry industry would be to focus on control options.

It's nice to know what Centers for Disease Control tell us about how many people are getting sick, it's nice to talk about a lot of the broader epidemiological things, where is it coming from, what's the problem like, how does it differ in Pennsylvania from, you know, et cetera, et cetera, but the bottom line is that especially looking at the climate that industry/government/consumers are living in in terms of how this problem is being approached by us as a society, and how we all have to respond to it, at the level of the industry sooner or later the bottom line for them I think is that they need concrete specific tools that will enable them to continue to do business in the face of this problem.

So I think the things that emerge there in my mind that address those kind of issues the most strongly, considering the type of broader approach we're likely to see in the form of perhaps a national SE control, or SE quality assurance, or whatever kind of program it will look like, but I think the kinds of things that are going to emerge there as Charlie highlighted refrigeration issues, egg storage issues are really a linchpin of a lot of these proposed control efforts.

Understanding what this is going to achieve, what it's not going to achieve, how we should do it, how we need to try to do it, et cetera, is really critical, so all of these issues that relate to how SE is deposited in eggs, where, when, how much, how it grows, how it is affected by refrigeration, how quickly eggs are cooled, how the SE do or do not grow under all these kinds of considerations, whether it does or does not, whether it's in the yolk, whether it's in the albumen, whether it can go from one to the other, whether the nutrients can go from one to the other, there's a host of questions that are all subsumed in that category of SE deposition in eggs and how that's affected by our proposed control regulations, because that's such a central part of all our proposed control strategies in virtually every direction, I think that one is really central.

The other area, and again I'm probably just reiterating what a lot of other people have said, in terms of what tools can be provided to a producer I think a solid understanding under field conditions of what vaccines, rodent control, cleaning and disinfection, feed treatments, all the types of things that are proposed as intervention strategies what they will do in application in commercial flocks is really, really critical information, and I think as Charlie is indicating information that's provided by a source other than the proponent, developer, or seller of these products, so that we, those of us that, you know, including in the research, regulatory, and industry communities all know which products, which interventions really are worth something when we try to use them in the field.

And third, and this one is maybe the least direct and least applied, but I think it affects what will happen in that area that I just mentioned, is actually getting down to -- and this is out of what I talked about earlier -- getting down to a hard and fast idea of what the real sources are, and we talk a lot about saying maybe it's laying houses that we're not cleaning and disinfecting, maybe it's rodents. Well, at some point one of the most practical ways to provide producers with the means to get out of this problem is to try to find a way for them to shut off the tap so that the next flock down the line isn't positive the way the last three have been.

And in addition vaccine is nice, vaccine may be -- if we ever get a perfect vaccine then maybe we could stop that. We don't have a perfect vaccine on the horizon, so in the meantime it would be really nice if we can have some idea where the real flow flock-to-flock is.

DR. WALTMAN: I share three possible areas. The first if, or maybe in this audience when this becomes a national mandatory testing program there is a need that the procedures be standardized, and in particular the sampling protocols that I mentioned earlier that we be able to standardize that across the country and from house to house.

The second is that it would be nice to have a more rapid detection method, but again that has to be specific for SE.

And then the final one is that as long as we're basing the diversion of eggs on the isolation of SE from the eggs we need to be able to do a better job of screening these eggs. If we could come up with some way of preselecting those eggs so that those thousand, or those five hundred that we are looking at better represent the possibility of getting contaminated eggs it would provide us better information.

MR. BRACKETT: Ahmed.

DR. YOUSEF: I have only one suggestion. The problem of natural versus artificial contamination, this will help us as a research tool if there is an artificial way of inoculating the egg with *salmonella* and that mimics the natural infection will make our life a lot easier. If not, then what else can we do.

I am just a microbiologist, so I don't really deal with live hens, and I usually ask veterinarians to provide me with naturally-contaminated eggs, but they have said that it's kind of difficult and very expensive, and there is no standard way of doing that, so if there is a way to standardize this and help microbiologists with either an easy way to produce naturally-contaminated eggs, or do artificial contamination in a way that is acceptable and mimics the natural infection that will be very, very helpful.

We just heard from Dr. Beard that *salmonella* is not inside the yolk, it is on yolk. At the same time FDA asked us to inoculate eggs inside the yolk otherwise our data are not valid, so where do we go.

MR. BRACKETT: Bailey.

DR. MITCHELL: I had a few points, some of which have already been mentioned in one way or another.

It seems to me that we could use some identification of some of the types of things that are done in the broiler industry in looking at critical control points. We tend to look at -- it seems from my perspective we're looking primarily from the production house out to the consumer and the various things that go on there.

Obviously those birds in that production house had to come from somewhere, so it seems to me that we need to get back and look at the breeder house as well, see what role that plays.

And the hatchery where you're hatching those eggs, and also in the production house. And then this sampling thing, it seems -- and I'm not a microbiologist, but I've been around enough of them long enough to get a feel for them -- it seems that --

DR. BEARD: Be careful, Bailey.

[Laughter.]

DR. MITCHELL: I should have said appreciation.

[Laughter.]

DR. MITCHELL: I think there are some possibilities that would be somewhat in the direction of what has been used in years past for virus sampling, you know, like a high-volume air sampler concept, except something that's a little more adaptable and user friendly. I think there are some possibilities there for sampling air within a house, and I'm fairly satisfied personally that that's going to be some fairly good representation of what's going on in a group of birds if you look at air. Birds will generate a tremendous amount of particulate matter whether or not they're on litter or not, and layers are no different, so they generate plenty of particulate matter, and if they're infected with SE they'll put it in the air without a problem.

So I think something along the line of a good high-volume air sampling that could be done maybe at the exhaust stream of a house as a means of assaying contrasted maybe to a drag-swab type thing, or compared to that.

I think too we need to put some effort into some different intervention strategies. A lot of the talk is about microbiological approaches. As an engineer I'm satisfied that there are some engineering interventions that can be done.

We've got houses at our lab where we raise disease-free birds, it's basically a combination of structural and air handling that we have been doing for many years, and you use portions of that concept without going to the extent that we do with concrete block buildings and high-efficiency filters and management. I think you can use portions of that as is done in some European countries and develop some good intervention in that way.

The other aspect of that is that not only could you use that to your benefit in controlling SE, but you probably are going to generally improve the health of the birds and the folks that are working in those buildings.

So that's all I have to offer.

MR. BRACKETT: Thank you. Peter.

DR. HOLT: I have delayed it long enough, I guess.

I think a lot of my feelings pretty much mirror what a number of the folks have already said, especially Richard and Bailey.

I think that more than anything else we need to find out what the source is. You know, the SE doesn't just appear by magic, it's coming from somewhere, so we need to figure out where and stop that.

We also need to develop better intervention strategies, i.e. more than anything else I think vaccination will probably be one of the biggies.

As I talked about this morning, competitive exclusion does have its functions, but I think that its functions are fairly early in the life of the bird.

And finally like Bailey was talking about with the type of situation you have to identify the risk factors that are exacerbating the problem, and not the least of which in my opinion is molting.

I think the reason I bring this up more than anything else is because, you know, I have been kind of caught in the middle of a lot of the controversy with, you know, my experimental data, and there isn't a lot of field data to go with it, and I really think that before very much longer, before somebody says that molting is a major risk factor for SE-positive eggs we definitely need to get a little bit more science-based information to say yea or nay on that, because it's a tremendous stretch to go from experimental data to the real world, and so I think that's really important to either, you know, bring it out in the open or put that baby to bed.

That's all I have to say.

MR. BRACKETT: Thank you.

Let me ask a reverse question. Is there anything that any of you have seen from the risk assessment, or excuse me, from the SE plan research that you think that we can put to bed, that has been done well enough that we don't need to be going down that route any more? This is a more difficult question sometimes.

DR. BEARD: Yes, there is one.

MR. BRACKETT: Charlie.

DR. BEARD: The one that I mentioned earlier was that in the initial stages of the problem there were people in the industry that did not believe a colonized hen could produce an internally-contaminated egg.

That has been put to bed. I don't think we need to go through that again. I think everyone will acknowledge that colonized hens can lay a percentage of contaminated eggs. That percentage we don't really know what the influence of the strain is. That would be very important, the strain and the influence of stresses another factor.

MR. BRACKETT: Okay. Anybody else?

The second question is -- again it's a difficult one, or maybe I shouldn't say difficult, but somewhat predictable in many cases -- is we have all these research priorities, some of which that I've heard here, particularly our vaccine development and intervention strategy as a top priority, one of the top priorities -- who is best to accomplish this?

Again, Charlie, you addressed this a little bit about not being the manufacturer, but this could come in one of different ways. Who should fund this so that it can be accepted scientifically? and how is this best accomplished in terms of the fusing? Is it competitive grants, or would it be directed contracts, private industry funding their own way? Do we have any opinions on that?

DR. BEARD: Bob, I would like to say that there are a lot of companies already using vaccine, they are very high on it, they rely on it when they're moving into a house that has tested out positive as the spent flock is being removed, the pullets going on there will be vaccinated.

It may just simply be that we need a researcher to go out there and work with the companies that are using it and collect the data, and it may not require a lot of funding, but the independent researcher can look at the response and monitor the flock and come to his own, his or her own conclusions. That would be my suggestion, take advantage of what's going on.

MR. BRACKETT: Okay. Jean.

DR. GUARD-PETTER: Let me just put a price tag on a simple vaccine trial of about $100,000 by the time you do the egg culturing, the organ culturing, the intestinal and environmental swabbing, and keeping the birds for X number of days in a laboratory that's about what I would charge to ever do one of those things again. It's quite labor-intensive, there's a lot of data requirements for recording results.

I'm having trouble conceptualizing if you're going out into a farm where they're just in production, are you going to culture the eggs, are you going to sacrifice a percentage of the birds and look in organs? I don't know, Peter, do you want to make some comment on going out to different farms and doing a vaccine trial?

DR. HOLT: It will be tough. You pretty much have to establish what kind of parameters you want to look at. I think you have touched on it very nicely there.

What do you define as protection? Is it a positivity? Is it, you know, a decrease in environmental positivity, organs, whatever? I think that needs to be established before you go much further.

Running a vaccine trial out in the field, I don't know. That would be tough. And I tend to agree with Jean as far as running one in the lab, it's a lot more labor intensive than it looks, and it does tend to be a little bit expensive, and I can't see where it would be any less expensive out in the field as well.

MR. BRACKETT: Richard Gast.

DR. GAST: This is following up on what Pete and Jean are talking about, and this actually is a funding -- even though it seems like we've strayed off of the funding question I think Bob asked initially, but not entirely because it does affect some of that rationale of how funding should be set up and how it should be allocated.

Bridging that gap between working in a lab environment like many of us do, and that's where money seems to end up going most of the time in our standard granting processes, it goes to some scientist who works in a laboratory environment for the government or for a university who does a research project that's usually done in their facility, I think we're all to some extent in agreement that although we think there's value to that, and although we have to think there's value to that presumably because it's much of what we do, I think you've heard as a common theme throughout much of the morning and the afternoon as we've talked about this the belief that the next level of research if it's going to really have impact on the problem is going to largely be done at the field level, and cooperation with commercial entities, collaboration both with the commercial entities that produce these intervention products, and more critically with egg producers is going to determine whether we're able to get this done.

It seems so obvious on paper, but it's not as easy when you try to take this in practice and go do it. Many of us have at one time or another, whether we be an individual researcher, or whether we be a large entity like the NAHMS survey, been in the process of trying to approach the industry to secure cooperation and participation to allow us to become involved.

This is a very politically and economically sensitive issue. Securing that cooperation is at times exceedingly difficult. Somewhere in the funding system -- and I don't know exactly how this affects how funding ought to be set up -- but something that would create projects that would have either enough demonstrable impact to the industry or enough critical mass, or some sort of clearly-perceived independence from bias that industry support could be secured, I think that's something the funding needs to think about. Just to throw money out and say if Professor Smith or Dr. Jones wants to look at this problem, that's not the same as making sure from the beginning that the whole thing is styled in such a way that we'll be able to ask the questions the way we need to ask them.

I think many of the questions we're talking about can only be asked in field settings with the active and rather extensive cooperation of people that own chickens.

MR. BRACKETT: Am I correct in understand you're saying that regardless of the funding agency that projects need to specify and be narrowly focused on field research only?

DR. GAST: No, I'm not trying to argue that.

MR. BRACKETT: Or individual projects I should say.

DR. GAST: I'm not trying to say that we should only do field research, nor am I saying that we ought to specifically fund only field research.

I'm saying that somewhere in how the funding is packaged it ought to be directed in such a way that enables the person who is going to do the research to be able to secure that cooperation, because I can propose -- as some of us know I can propose to do something with industry, I can even go perhaps and convince a funding agency that this is worthy of being done, and they may even write the check and hand it to me, but if I can't get XYZ Eggs, Incorporated to say "Okay, you can come into our houses and sample," or "We'll do the samples and provide you the samples," or the data, or whatever, and I see that as a major barrier, and I don't know if it's a question of how money is allocated.

I admit this is only tangentially touching on this issue of where the funding ought to come from and how it ought to be set out, but I think before money is set out there ought to be some clarity of ability to get done the mission that the money really needs to support.

DR. BEARD: That ought to be part of --

MR. BRACKETT: Charlie, could you use the microphone, please.

DR. BEARD: Richard, that needs to be part of the preparation of the proposal. We get a lot of proposals into our association, and those proposals are the result of discussions between researchers and companies, and that's all worked out and is documented in the proposals, so you're not going to get any funds unless you have the company identified that's going to participate, and at what level it's going to participate, et cetera. So that can and has been done with other issues.

As far as the criterion upon which you would judge the efficacy of a vaccine, I vote for rate of egg contamination. That's what we're dealing with here.

DR. YOUSEF: If there is a specific problem that deals with a company or a group of companies, of course I go to these companies directly and ask them for funding. If the problem is wider then I go to the trade association and ask them for funding.

If the problem cannot be funded by the industry or the association, I think the government is paying for that, something like an issue that the industry doesn't like to address, or the industry is not willing to address at this point, something related to safety of consumers that need to be revealed, and then I would say the FDA will be helping with this kind of thing.

I was tempted to say FDA will fund things with match from other places, but I decided not to do that, but I said it anyway.

MR. BRACKETT: Okay. Rather than the FDA what you're saying is government funding who actually has the money.

Eric, did you have a comment?

DR. EBEL: Yeah, I guess I do have a comment, and I wanted to remember back to 1992 when the Pennsylvania Pilot Project was launched in collaboration with the two universities in Pennsylvania as well as the state Department of Agriculture and USDA, and unfortunately it seems to me that most of the field evidence we speak of today came from the first two years of that project, yet the work continues in Pennsylvania, and one of the things I think would be very helpful in answering many of these questions, or continuing to answer the questions that began to be addressed by that pilot project is to put more analytic resources into the

Pennsylvania Egg quality Assurance Program. I think that is an established program.

We see out of the Pennsylvania Pilot Project issues link vaccination efficacy, there was some beginnings of analysis of that. Certainly the question of molting and the effect on egg contamination frequencies was addressed by that pilot project, so a whole host of many of the issues that have been brought up by this panel I think are things that can be addressed in the context of the Pennsylvania program as it currently exists, or can be added onto it if there were additional resources and an agreement to do that, and/or other programs throughout the country.

I think one of the problems that we all recognize is that simply relying on the largess of Pennsylvania is both maybe inequitable as well as not representative of the entire industry, so the idea of expanding that kind of support to other programs and activities in the egg industry I think would be useful.

MR. BRACKETT: Doug.

DR. WALTMAN: Thank you.

Over the last ten years we have basically placed the burden on the industry itself, and I know many researchers have done some really nice work on very low budgets, and it's matter of us pulling from here and scrimping there, and a lot of the reasons we don't have more answers today is that there really wasn't a lot of funding available for us to seek out those things.

Along those same lines, if we are putting in a mandatory program for the egg industry I think it would behoove FDA or the government if nothing else as a good will gesture to say we will put in this much money for research in trying to better define and to better deal with this issue, so I would love to see the government do their part.

MR. BRACKETT: Jean.

DR. GUARD-PETTER: One thing that I think it's possible for the government to do -- there would have to be some consensus with industry, producers, and the researchers -- is perhaps fund a standard challenge to measure egg contamination, because we do have the experimental models where we can get the hens to at least contaminate eggs via the reproductive tract, not injecting the eggs, and then if vaccines from different sources were plugged into the model and the challenged strain kept standard, the age of the bird kept standard, we could at least compare the vaccines for very specific things like egg contamination, and maybe organ invasion.

Now, is this predictive of how the vaccine is going to be in the field? Not necessarily, but it would be at least some sort of comparison, but if the money comes from industry I don't see how they will ever go along with it.

MR. BRACKETT: Richard.

DR. GAST: Just to follow up on what I was saying before now that I've had a chance to think about your question, I would say that in fact a part of what I would be arguing for would be indeed that some proportion of research funding ought to be specifically earmarked for field research so that it's by definition requiring investigators to set up the kind of collaborative things that are necessary to get the mission done, and I would also add that I think Eric is making good sense as well, but we ought not to be reinventing the wheel.

If we have a mechanism available for data collection -- I mean a concrete example of when in some of my own experience trying to secure cooperation from industry for proposed experimentation -- for example, people in Pennsylvania have made the argument justifiably "Why should we work with you, we are already working with our own program." If we in fact have large sets of producers as is the case in Pennsylvania already willing to work with investigators to gather some of this kind of information, and if we have already got an apparatus in place to do some of that we would be very foolish I think not to take advantage of that.

MR. BRACKETT: Anyone else from the panel? Yes, Bailey.

DR. MITCHELL: I just wanted to comment on the funding issue. I have been involved in some field studies the last two or three years in working with various companies, and one thing that appears to me is that if we do field studies in the future with SE it's going to take some money. You don't just grab folks, you know, off the street and run them out there and make assays and do field surveys. It takes trained people, and you can only do so much. You know, it takes folks, it may take some extra hands, some grad students and post-docs and what have you, and then the lab work you can't always depend that a company is going to have sufficient laboratory facilities to do all that stuff, so there's going to be some expense involved in that. So I think definitely some funding is needed, and probably a lot more than is floating around right now.

And I kind of like Doug's comment even though I'm a government man for a long time, it does seem appropriate that if you're going to impose regulations and expect a quick response by the industry that the appropriate, they have a good bit of funding coming in support of that for the research that's needed.

MR. BRACKETT: Any other comments from the panel?

[No response.]

MR. BRACKETT: At this point since the panel has had their opinions I wanted to offer the opportunity to answer those questions from the audience as well just very briefly. We have about ten minutes before the public comment period in which any other issue related to the topic today can be discussed.

Does anybody else have an opinion as to what the priority research should be, and how this is best accomplished in terms of who funds it? And could you please use the microphone, state your name and affiliation, please.

MS. CURTIS: Pat Curtis, North Carolina State University, and I just have one comment regarding the research from the process when we were talking about time, temperature, storage issues.

One thing that hasn't been brought up is nest run eggs, and when we start looking at those as compared to the in-line or off-line operation eggs I don't know if we have a survey that tells us what percentage of eggs are nest run, what's the average age of nest run, but the reason I ask this question is that at North Carolina State we do a lot of our research in the field, and I actually had a whole experiment that I had to repeat because I got a hold of some nest run eggs that happened to be three weeks old before they were processed, and it messed up everything else because when I added that three weeks to what I was doing, so I don't know if that's uncommon or whatever, but I've said that if you're going to look at research on storage you need to make sure that you really know what that date is on that storage that you're looking at.

MR. BRACKETT: Jill, do you have a question on those, or a comment on those questions?

MS. SNOWDON: I'm going to reserve the bulk of my comments for the public period because I thought that was my time slot, but I did want to be supportive of your question of how to fund, and I'm going to encourage a mixture of routes in terms of the way to go so you've got some balance in terms of your funding mechanisms using your resources that you've got at hand through government structured programs, and yet at the same time tapping into other resources and innovation outside of government researchers so that you get that kind of input also, so a mixture of your funding practices I think are going to give you your greatest yield overall.

And a comment not so much for this program, but as much as what I've seen with federal research attitudes on food safety, and that is that we spend years identifying research gaps, and so I have just come from a conference that happens once every five years, and we're again talking about research gaps, and we say "How about if we talk about what we did over the last five years?" instead of more gaps, so I certainly want to encourage, be supportive of moving from identifying goals to making sure the projects get funded and the research gets done and then published also. Getting it published is an important part of the whole process, that we're not talking about what somebody did, or said they did five years ago, but that we can all know about it and move in with it.

And then part and parcel of the mechanisms of funding I think we have to think broadly that it is a nationwide problem with distinct geographic pockets, and that there has been research going in a variety of states looking at that, and that if we use any one state or geographic area as a model for the entire country I think that's limiting, so those three concepts of diversity really, and action in terms of how we proceed on the funding.

DR. BEARD: Bob, could I make one comment while he's on the way to the mike?

MR. BRACKETT: Yes, Charlie.

DR. BEARD: As far as funding research on SE, I administer a research grants program for the U.S. Poultry & Egg Association, and there has already been a lot of industry money going into SE research. We funded the SE egg cooling studies that Dr. Curtis spoke about, we have funded her studies she spoke about, we are currently funding another study at North Carolina State on egg cooling, we have funded Dr. Holt's molting research. We have funded a lot of SE research. The problem is we don't get many proposals.

MR. DENUDE: I'm Greg Denude, I'm with the FDA at the National Center for Food Safety and Technology outside Chicago, I just want to say to address Dr. Waltman's comments about that we should be putting more FDA or giving more government funding I just wanted to say that I'm starting a project that's being supported by the FDA concerning microwave sterilization, or in-shell pasteurization using microwaves, microwave energy, and we're going to be starting that in October, so there is a little bit going on.

MR. KINDAY: I am Hidro Kinday from University of California at Davis, the diagnostic laboratory system.

I just have one comment for the panel in regard to research needs. Through the years at least for the last ten years data has been gathered throughout the country by different diagnostic laboratories and industry. I wonder, we can analyze these data in cooperation with the industry and see what has been done. The industry has -- some of the ones I know they have excellent record, production record, testing record, and even molting, we have been talking about molting. Molting is a common practice in the industry, and we can factor in all this and see where do we need to go from here, and this is an excellent really resource to look at it, and we don't need to reinvent really the wheel. Data has been gathered there, and it's a matter of analysis and seeing in what ways can we supplement to the industry so that they can do the best.

MR. BRACKETT: It's just about time for the public comment period to begin, and what I'll do is tell all of our panelists, thank them all for their participation up here, and they don't have to sit up here any longer unless they really want to, but during this comment period this is a time that is reserved for any public comment on the particular topic that we have been discussing the whole day, that is research on *salmonella enteritidis* as it applies to the action plan, and we do have some requirements

First of all, the comments may not be any more than five minutes. However, if you have written comments either with you today, or if find in the next few weeks or months that you want to submit written comments in your packet is an address that you can submit those to Dockets and make it official afterwards as well.

And along with that all of the discussions here today, the materials that have been provided to us as far as visuals, as well as the public comments will be available from Dockets after thirty days, and so can request transcripts and materials that way as well.

We will address the comments at this time, and what we will do is call those individuals who have signed up to give public comment first, and then when they are through if anybody else has a comment we will be happy to entertain that as well.

The first one we have is Jill Snowdon who has asked to give her public comment.

And again as each person comes up there, would you please state your name and your affiliation, please.

STATEMENT OF JILL SNOWDON, EGG NUTRITION CENTER

MS. SNOWDON: Thanks, Bob. Jill Snowdon with the Egg Nutrition Center.

I've got a tremendous amount of support for the items that have already been identified that I think we've got the breadth and depth of things and yet some focus also.

In particular detection technologies so that the ability to predict the house, the flock, or the egg, or the human at risk I think is one of the most important things, so however we go about that that ability to predict the house, flock, egg, or human at risk I think is the priority item relative to what we have been speaking about.

But in addition to the things that have previously been talked about I would like to add a couple of things, and I call them the simple and the social.

The first one is a little bit tough to explain, the simple, in that I think we still need to build a better mousetrap, that rodent control is one of the most important control points in all of this, and yet we don't necessarily have rodent control out on the production facilities.

Well, why not? And what's it going to take to get it there? And is that social research into the behavior of producers? or is that an easier and better mousetrap?

So cheaper, faster, easier to implement, easier to manage is always better in a production environment, and likewise with cleaning and disinfection. So these are things that we've identified as risk factors in support of and controlling the programs and a challenge to what extent we know they're implemented and being implemented with the level that they need to be implemented.

So I call that the simple in that it's not highly sophisticated or complicated, but still needs I think work on that.

And when I talk about the social we have kind of concentrated an awful lot on the production environment on this, and with the exception of Christine's presentation which was funded through the California Egg Commission when I was a consultant with that group, and so it again is reflecting the need to start looking at things beyond the production level and how we get the job actually done, and so from the social viewpoint of research whether it's talking about motivating a producer, or motivating the consumer, or motivating the food service worker that I think there are research opportunities there that those of us that tend to work in the biological sciences don't identify or discuss because they're not our bailiwick.

And that goes the same for epidemiology, human epidemiology and understanding. We've got data from CDC on *salmonella* systems that's talking about who's getting sick from what when, and yet that's not necessarily being applied, and we've got 25 percent of our SE cases are in those under age ten, and yet we constantly hear the refrain we've got to have pasteurized eggs in nursing homes. Well, we do have to protect the elderly, but, you know, if 25 percent is under age ten then they're not going to be addressed by that particular thing.

So tying the epidemiological, the human epidemiological data into the whole process I think is an important research area.

Likewise with risk communication, what messages need to be communicated, how to communicate them with the goal of changing human behavior, which is some of the things that Christine Bruhn was talking about.

So the simple and the social is the short version of what I'm trying to put forward, to build the better mousetrap and then get the word out where you need to get the word out as to how to get things done so that the disease level in humans keeps dropping.

Thank you.

MR. BRACKETT: Thank you, Jill.

You may take the microphone and state your name and your affiliation. You're next.

STATEMENT OF KAREN DAVIS, UNITED POULTRY CONCERNS

MS. DAVIS: My name is Karen Davis, and I'm the president of an organization called United Poultry Concerns which is a national nonprofit organization addressing the treatment of chickens and other domesticated fowl.

I want to tell you quickly a few things about myself so that you understand where I am coming from at this meeting. We represent the welfare of the birds, and I know that most of you are very aware that there have been some major announcements from McDonald's about changes they're going to require of their suppliers of eggs, and I have to tell you that the issue of forced molting in particular and the case for chickens you may attribute to me in the animal welfare community because I brought the practice of forced molting to the attention of our community which was unaware of it until I dug it up in the early 1990s and made a huge issue of it with People for the Ethical Treatment of Animals and all of the other organizations who have now taken off with the information that I found at the Beltsville library, and through all the journals and industry magazines that I subscribe to through our organization, and the various conferences I have attended over the years.

I have focused upon forced molting, and I have succeeded in getting the attention of the animal protection community to such an extent that we see this major announcement by McDonald's, and I know how important United Poultry Concerns has been behind the scenes of everything that is taking place.

I drew attention to the forced molting as a cruelty and inhumane issue initially, and then through my further research at the Beltsville library I encountered the laboratory studies of Peter Holt and some others showing a probable causal connection between the practice of forced molting that entails food withdrawal, sometimes water withdrawal, immune system dysfunction, and consequent *salmonella enteritidis* in the hens' ovaries, oviducts, intestines, in eggs, et cetera.

And I guess I'm here to say partly we have, or organization jointly with another national veterinary organization submitted a citizen comment to the Food and Drug Administration in April of 1998, and it was a very comprehensive 16-page fully documented citizens' petition -- excuse me, citizens' petition requesting that the Food and Drug Administration use its mandate to intervene when farm practices have been shown to be a probable cause of a human health problem, and we have not been pleased with the response so far that we have received from the Food and Drug Administration to date in acting upon our petition.

This does not mean that I have no hope, or that I have, you know, that I'm completely disappointed. That would not be true. I was very pleased to have a meeting last week with several administrators, some of whom are here today at my request which they so graciously granted.

But I can tell you this: That I continue to read up on this subject of forced molting in addition to other welfare issues, and I write extensively, and I wrote a book about the poultry industry, the poultry and egg industry, *Prisoned Chickens, Poisoned Eggs, an inside look at the modern poultry industry*, which has really become a kind of bible in our community because all the information is taken directly from the industry and from those adjunctive scientific researches, and I'm being told now that it's my turn to close my comments.

But I do want to say this: We would like to see the Food and Drug Administration, the U.S. Department of Agriculture Food Safety Inspection Service, and everybody else involved, all the researchers here to do the thing that we are asking, and I can assure we're not going to go away, we are going to continue to amplify the issue before the public. The question has been raised here coming from another angle, how do you motivate the public. I think one thing we're beginning to see is that our articulate ability to articulate what is being done to these birds, and forced molting is only one of those things, that leads to the contamination problems that have been identified is not something that we're going -- we're not going to rest on our laurels now that we have got some kind of lip service which we hope will be more than lip service, and I believe it is, from McDonald's. We want the public to know how the birds are being treated, and what is done to them as biological organisms with a psychology and many, many functions analogous to ourselves causes as in ourselves when we are in slum conditions and similar conditions illness, and illnesses which in some cases, and perhaps in some, many even ultimately untraceable cases --

MR. BRACKETT: Ms. Davis, I'm sorry --

MS. DAVIS: -- can be passed on to humans.

MR. BRACKETT: -- your time is up. We'll have to --

MS. DAVIS: My time is up, but I want to emphasize this: Our organization United Poultry Concerns has attended, either I myself have attended, or a representative has attended every single egg safety meeting, and we want to see research that is really going to lead to an end to some of the practices, and in particular forced molting I mention right now, but that's not the only one, that lead to the kinds of diseases that are being discussed here and that our great concern is that would be treated with technological fixes that do not address the fundamental core being who happens to be the bird.

Thank you.

MS. DAVIS: Thank you.

MR. BRACKETT: And again, please, if you have additional comments we do invite you to submit written comments as well either today or later.

Is there anyone else who would like five minutes for a public comment?

Yes. Please state your name and your affiliation at the microphone.

STATEMENT OF PHYLLIS BEDFORD, PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

MS. BEDFORD: Good afternoon. My name is Phyllis Bedford, and I represent People for the Ethical Treatment of Animals.

Please accept the following comments on behalf of PETA's more than 700,000 members and the more than 234 million hens who endure the cruel practice of forced molting every year.

Any effective egg safety action plan must address *salmonella enteritidis*, SE infections, at their source. Sadly the President's Council on Food Safety's Egg Safety Action Plan fails to adequately address one of the most significant causes of SE, a practice commonly referred to as forced molting, despite overwhelming evidence that this practice results in an increased frequency and severity of SE infections in laying hens.

In order to effectively reduce the hazards of SE it is absolutely critical to eliminate this specter of transmission. We therefore urge the FDA and FSIS to include a strict prohibition of the dangerous practice of forced molting when the egg safety regulations are written.

Scientists have shown both in the field and in the laboratory that forced molting leads to higher rates of SE and, as a result, causes serious human illnesses which can potentially lead to death.

For example, the U.S. Department of Agriculture recently reported that the number of human SE infections would be significantly reduced if forced molting were eliminated. Even the USDA's Food Safety and Inspection Service advises that, quote: In an effort to reduce human illnesses caused by SE, FSIS is encouraging poultry and egg producers to eliminate forced molting practices, end quote.

Another USDA study concludes that forced molting increases the frequency and severity of SE infections in a flock and, quote: could conceivably alter the SE situation in a flock from a minor problem involving a small number of birds to one where a large number of birds are affected, end quote.

Similarly, a study out of the University of Florida finds that the stress caused by a forced molt significantly compromises the immune system of laying hens, resulting in higher levels of SE infection. The study concludes, quote: Molted birds showed significantly higher numbers of SE during a forced molt as compared to unmolted birds, and forced molting causes an increase in the transmission of SE to uninfected hens housed in adjacent cages, end quote.

These studies are only a sample of many in existence pointing towards the dangerous implications forced molting has on both animals and human health. The Food Animal Concerns Stress in the United States also reports that by using systems that preclude forced molting in layers SE was reduced by up to 70 percent, and the top consumer groups in the U.S. have taken a strong stance against the practice due to the serious health risk it creates for consumers, including the Center for Science in the Public Interest, Consumers Union who publish the *Consumers Report*, and Public Citizen.

Perhaps the greatest hardships caused by forced molting, however, are to the hens themselves. This inhumane practice inflicts intense and unjustifiable suffering for more than 234 million hens each year by starving them for up to two weeks often in darkness. Hundreds of thousands of die, while those who survive shed their feathers, lose up to 35 percent of their body weight, and grow weak.

The stressful conditions weaken the birds' immune system so badly to the point where -- Excuse me, I'm sorry. It hurts their immune system to the point where they become prone to disease, especially SE infections. The result is sick birds and contaminated eggs.

Any one of the nearly four million infected eggs produced every year in the U.S. can cause a dangerous outbreak that can affect hundreds of individuals. It is therefore imperative that the SE infection be prevented and addressed in the hen at the source of the problem by explicitly prohibiting the practice of forced molting.

The serious risk to human health and to animal welfare caused by forced molting can no longer be ignored. The occurrence of fatal SE poisonings and severe animal suffering caused by the practice are all to real.

Once again, on behalf of our members we urge the relevant agencies to adopt specific language prohibiting forced molting in egg safety regulations, and, as a result, help reduce animal suffering, human illness, and taxpayer medical costs.

Thank you for the opportunity to comment.

MR. BRACKETT: Thank you for your comment.

Is there anyone in the audience in addition?

Yes.

STATEMENT OF CHUCK BENSON, UNIVERSITY OF PENNSYLVANIA

DR. BENSON: I couldn't pass the opportunity of an open mike. I'm Grandfather Benson from the New Bolton Center School of Veterinary Medicine of the University of Pennsylvania.

To my knowledge we were the first ones to isolate SE from an ovary, and subsequently SE from eggs from that same flock, and I just wanted to share a few thoughts about the meeting.

I have been to a number of these ever since 1987, and the questions are almost always the same. Sometimes it looks like we move forward, and sometimes we don't.

From my point of view, and I'm trained as a microbiologist/biochemist with postdoctoral experience some millennium ago in biochemical genetics -- and this is not what I intended my life to be -- is that sensitivity of the testing method is lacking, and I need to share with you that the night Dr. Akrod at the University of Pennsylvania got a call and said "Will you test some ovaries?", he said "Yes," he transferred the call to me because I do the work, and they said "If you'll test these ovaries we'll have them to you tomorrow," so I spent four hours researching a technique, talking to Glenn Snellinbus who is since departed, Everett Bryant who is departed -- and I'm not sure that there's a relationship here -- Nelson Cox, I called Charlie Beard, and from -- and I read the books that AAP put out and devised a technique that showed that 62 percent of the ovaries they sent us were positive, but there was no scientific documentation at that time.

From that the techniques have evolved. There has never been good documentation for that.

The next comment I wanted to make was about molting, and to say that I'm not aware of any published study of a naturally-infected flock -- Dr. Benson, I should be about the fourth or fifth one down -- that has been molted and studied at that time. We have done that. I am not aware, I did not get the same results that Peter Holt did.

I was discouraged from publishing it by a couple of friends because it didn't jibe with what Peter saw. We saw no increase in SE secretion in eggs. We did see that the rooster became more virile -- I'm not quite sure what that meant -- and we observed the chickens for a period of fourteen weeks after the molt had ceased. They never at any time appeared distressed or unhappy with what they were doing, and I would encourage that a lot of the people in this room to need to get out into the field and see what's really going on, and maybe walk through that mature pit once. If you do it once you learn that you hire people who do it for you.

And so I would encourage the people who lobbied against molting to take into account that those are experimentally inoculated flocks, those are not real flocks, and I would question who and what ones of us can judge what really is cruel.

And I think those are my comments. Thank you.

MR. BRACKETT: Thank you very much.

Yes.

STATEMENT OF ROBERTA MORALES, RESEARCH TRIANGLE INSTITUTE

MS. MORALES: I'm Roberta Morales with the Research Triangle Institute in North Carolina.

I just wanted to bring up none research area that I have not really heard mentioned yet as of today, and I think it's an important area for an industry that has fairly small profit margins, and that is that I think we need more cost studies, both cost-benefit studies and cost effectiveness studies.

The issue of interventions and effective interventions have been brought up, but I think one thing that we need to be looking at is which of those interventions are really going to give us the best bang for our buck. We do have limited resources and moneys to put into any one area, and I think doing the cost-effectiveness or cost benefits studies would be a way to target where to put our efforts at and our resources.

A couple of things that were mentioned earlier were the epidemiological field studies which Richard Gast and a couple of other folks mentioned. I think those are important.

Eric Ebel had mentioned the intersection between the importance and uncertainty in identifying what are the risk factors that fit in that intersection, and I think the cost studies are another layer that says "Okay, we now have identified through field studies, epidemiological field studies what's important. We have also looked at where are we going to best be able to get the information on that from that intersection of importance and uncertainty."

I think the last layer to that is to say can we do the cost studies and get the information that tells us where we want to really allocate our resources and where we get the biggest benefit for what we put in, the investment.

MR. BRACKETT: Thank you.

Any other individual who would like to make a five-minute comment for the record?

MS. DAVIS: Can I make a one-minute comment?

MR. BRACKETT: No. Once you have made your five-minute comment we limit it to that, but we would appreciate our written response.

Is there anybody else?

[No response.]

MR. BRACKETT: Okay. Well, with that I would like to thank you all for your attention today, and especially thank our speakers who volunteered in some cases, and others were asked to come here and speak to this issue. We really do appreciate their participation, and we particularly appreciate the audience participation in coming here and listening to this, and providing comments as well.

This is part of a process that we go through in the regulatory agencies to get all aspects of an issue out, and we do appreciate it.

At this point I will conclude the meeting.

[At 3:50 p.m., Friday, September 8, 2000, the meeting was concluded.]

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REPORTER'S CERTIFICATE

DOCKET NO.: N/A

CASE TITLE: SALMONELLA ENTERITIDIS RESEARCH

HEARING DATE: September 8, 2000

LOCATION: Hapeville, Georgia

I hereby certify that the proceedings and evidence are contained fully and accurately on the tapes and notes reported by me at the hearing in the above case before the

Date: September 8, 2000

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